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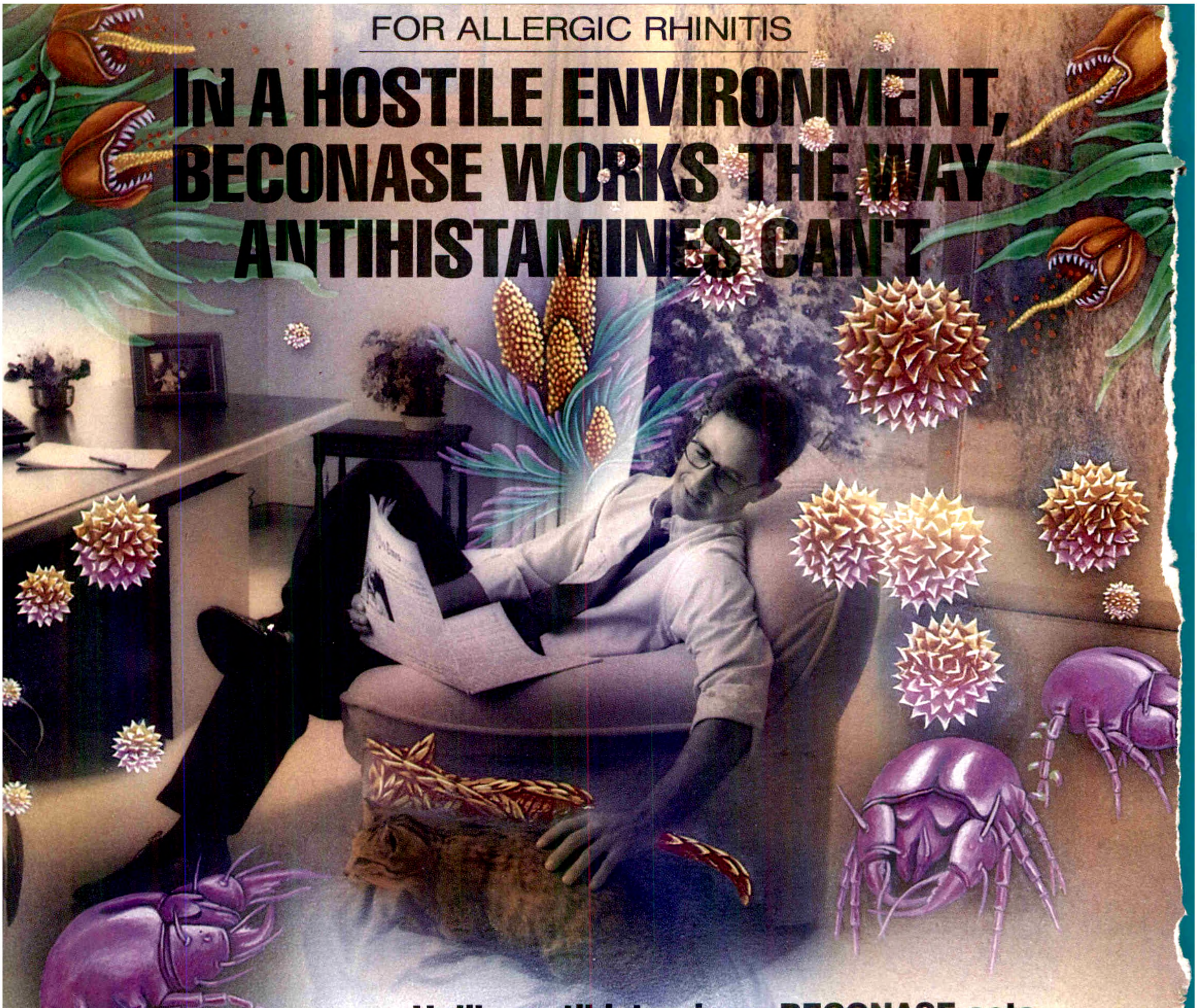

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Please consult references and Brief Summary of Prescribing Information on next page.

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BEFORE USE.

The following is a brief summary only. Before prescribing, see complete prescribing information in Beconase® Inhalation Aerosol and Beconase AQ® Nasal Spray product labeling.

CONTRAINDICATIONS: Hypersensitivity to any of the ingredients of either preparation contraindicates its use.

WARNINGS: The replacement of a systemic corticosteroid with Beconase® Inhalation Aerosol or Beconase AQ® Nasal Spray can be accompanied by signs of adrenal insufficiency.

Careful attention must be given when patients previously treated for prolonged periods with systemic corticosteroids are transferred to Beconase Inhalation Aerosol or Beconase AQ Nasal Spray. This is particularly important in those patients who have associated asthma or other clinical conditions where too rapid a decrease in systemic corticosteroids may cause a severe exacerbation of their symptoms.

Studies have shown that the combined administration of alternate-day prednisone systemic treatment and orally inhaled beclomethasone increases the likelihood of hypothalamic-pituitary-adrenal (HPA) suppression compared with a therapeutic dose of either one alone. Therefore, Beconase Inhalation Aerosol and Beconase AQ Nasal Spray treatment should be used with caution in patients already on alternate-day prednisone regimens for any disease.

If recommended doses of intranasal beclomethasone are exceeded or if individuals are particularly sensitive or predisposed by virtue of recent systemic steroid therapy, symptoms of hypercorticism may occur, including very rare cases of menstrual irregularities, acneiform lesions, and cushingoid features. If such changes occur, Beconase Inhalation Aerosol and Beconase AQ Nasal Spray should be discontinued slowly consistent with accepted procedures for discontinuing oral steroid therapy.

PRECAUTIONS:

General: During withdrawal from oral steroids, some patients may experience symptoms of withdrawal, e.g., joint and/or muscular pain, lassitude, and depression.

In clinical studies with beclomethasone dipropionate administered intranasally, the development of localized infections of the nose and pharynx with *Candida albicans* has occurred only rarely. When such an infection develops, it may require treatment with appropriate local therapy or discontinuation of treatment with Beconase® Inhalation Aerosol or Beconase AQ Nasal Spray.

Beclomethasone dipropionate is absorbed into the circulation. Use of excessive doses of Beconase Inhalation Aerosol or Beconase AQ Nasal Spray may suppress HPA function.

Beconase Inhalation Aerosol and Beconase AQ Nasal Spray should be used with caution, if at all, in patients with active or quiescent tuberculous infections of the respiratory tract; untreated fungal, bacterial, or systemic viral infections; or ocular herpes simplex.

For either preparation to be effective in the treatment of nasal polyps, the aerosol or spray must be able to enter the nose. Therefore, treatment of nasal polyps with these preparations should be considered adjunctive therapy to surgical removal and/or the use of other medications that will permit effective penetration of these preparations into the nose. Nasal polyps may recur after any form of treatment.

As with any long-term treatment, patients using Beconase Inhalation Aerosol or Beconase AQ Nasal Spray over several months or longer should be examined periodically for possible changes in the nasal mucosa.

Because of the inhibitory effect of corticosteroids on wound healing, patients who have experienced recent nasal septum ulcers, nasal surgery, or trauma should not use a nasal corticosteroid until healing has occurred.

Although systemic effects have been minimal with recommended doses, this potential increases with excessive doses. Therefore, larger than recommended doses of Beconase Inhalation Aerosol and Beconase AQ Nasal Spray should be avoided.

Beconase Inhalation Aerosol: Rare instances of nasal septum perforation have been spontaneously reported.

Rare instances of increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids.

Beconase AQ Nasal Spray: Rarely, immediate hypersensitivity reactions may occur after the intranasal administration of beclomethasone.

Extremely rare instances of wheezing, nasal septum perforation, and increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids. Although these have not been observed in clinical trials with Beconase AQ Nasal Spray, vigilance should be maintained.

If persistent nasopharyngeal irritation occurs, it may be an indication for stopping Beconase AQ Nasal Spray.

Information for Patients: Patients being treated with Beconase Inhalation Aerosol or Beconase AQ Nasal Spray should receive the following information and instructions. This information is intended to aid in the safe and effective use of this medication. It is not a disclosure of all possible adverse or intended effects. Patients should use these preparations at regular intervals since their effectiveness depends on their regular use. The patient should take the medication as directed. It is not acutely effective, and the prescribed dosage should not be increased. Instead, nasal vasoconstrictors or oral antihistamines may be needed until the effects of Beconase Inhalation Aerosol or Beconase AQ Nasal Spray are fully manifested. One to 2 weeks may pass before full relief is obtained. The patient should contact the physician if symptoms do not improve, or if the condition worsens, or if sneezing or nasal irritation occurs. For the proper use of either unit and to attain maximum improvement, the patient should read and follow carefully the patient's instructions section of the package insert.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of rats for a total of 95 weeks, 13 weeks by inhalation and 82 weeks by the oral route, resulted in no evidence of carcinogenic activity. Mutagenic studies have not been performed.

Impairment of fertility, as evidenced by inhibition of the estrous cycle in dogs, was observed following treatment by the oral route. No inhibition of the estrous cycle in dogs was seen following treatment with beclomethasone dipropionate by the inhalation route.

Pregnancy: ***Teratogenic Effects: Pregnancy Category C:*** Like other corticoids, parenteral (subcutaneous) beclomethasone dipropionate has been shown to be teratogenic and embryocidal in the mouse and rabbit when given in doses approximately 10 times the human dose. In these studies, beclomethasone was found to produce fetal resorption, cleft palate, agnathia, microstomia, absence of tongue, delayed ossification, and agenesis of the thymus. No teratogenic or embryocidal effects have been seen in the rat when beclomethasone dipropionate was administered by inhalation at 10 times the human dose or orally at 1,000 times the human dose. There are no adequate and well-controlled studies in pregnant women. Beclomethasone dipropionate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully observed.

Nursing Mothers: It is not known whether beclomethasone dipropionate is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised when Beconase Inhalation Aerosol or Beconase AQ Nasal Spray is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children below 6 years of age have not been established.

ADVERSE REACTIONS: In general, side effects in clinical studies with both preparations have been primarily associated with irritation of the nasal mucous membranes.

Rare cases of immediate and delayed hypersensitivity reactions, including urticaria, angioedema, rash, and bronchospasm, have been reported following the oral and intranasal inhalation and administration of beclomethasone.

Beconase® Inhalation Aerosol: Adverse reactions reported in controlled clinical trials and long-term open studies in patients treated with Beconase Inhalation Aerosol are described below.

Sensations of irritation and burning in the nose (11 per 100 patients) following the use of Beconase Inhalation Aerosol have been reported. Also, occasional sneezing attacks (10 per 100 adult patients) have occurred immediately following the use of the intranasal inhaler. This symptom may be more common in children. Rhinorrhea may occur occasionally (1 per 100 patients).

Localized infections of the nose and pharynx with *Candida albicans* have occurred rarely (see PRECAUTIONS).

Transient episodes of epistaxis have been reported in 2 per 100 patients.

Rare cases of ulceration of the nasal mucosa and instances of nasal septum perforation have been spontaneously reported (see PRECAUTIONS).

Rare instances of increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids (see PRECAUTIONS).

Systemic corticosteroid side effects were not reported during controlled clinical trials. If recommended doses are exceeded, however, or if individuals are particularly sensitive, symptoms of hypercorticism, i.e., Cushing's syndrome, could occur.

Beconase AQ® Nasal Spray: Adverse reactions reported in controlled clinical trials and open studies in patients treated with Beconase AQ Nasal Spray are described below.

Mild nasopharyngeal irritation following the use of beclomethasone aqueous nasal spray has been reported in up to 24% of patients treated, including occasional sneezing attacks (about 4%) occurring immediately following use of the spray. In patients experiencing these symptoms, none had to discontinue treatment. The incidence of transient irritation and sneezing was approximately the same in the group of patients who received placebo in these studies, implying that these complaints may be related to vehicle components of the formulation.

Fewer than 5 per 100 patients reported headache, nausea, or lightheadedness following the use of Beconase AQ Nasal Spray. Fewer than 3 per 100 patients reported nasal stuffiness, nosebleeds, rhinorrhea, or tearing eyes.

Extremely rare instances of wheezing, nasal septum perforation, and increased intraocular pressure have been reported following the intranasal administration of aerosolized corticosteroids (see PRECAUTIONS).

OVERDOSAGE: Information concerning possible overdosage and its treatment appears in the full prescribing information.

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PREEPIGLOTTIC SPACE INVASION IN "EARLY" EPIGLOTTIC CANCER

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The tendency of epiglottic cancer to invade the preepiglottic space and the significance of this invasion was evaluated in 36 surgical specimens of epiglottic carcinoma originally staged T1 or T2. None of the 9 lesions originating above the hyoepiglottic ligament invaded the preepiglottic space. Twenty-four of the 27 (89%) lesions originating below the hyoepiglottic ligament showed invasion of the preepiglottic space and were clinically understaged. Of the 3 infrahyoid lesions showing no invasion of the preepiglottic space, 1 was a verrucous carcinoma and another was a mucoepidermoid carcinoma arising on the petiole. All other lesions were squamous cell carcinoma. In 12 of the 24 patients (50%) with preepiglottic space invasion, cervical node metastasis was present despite the preoperative staging of T1 or T2.

KEY WORDS — cancer, epiglottis, larynx, preepiglottic space, staging, supraglottis.

INTRODUCTION

From his analysis of whole-mount laryngeal sections in 1936, Leroux-Robert[1] described the difficulty in clinically assessing the depth of invasion of lower epiglottic and ventricular fold cancers. He also explained that these cancers were predisposed to invade the preepiglottic space. In 1940, Clerf[2] reinforced these concepts and in 1944 he further stated that lymphatic drainage from the base of the epiglottis is carried across the preepiglottic space through the thyrohyoid membrane to the upper deep cervical nodes.[3]

Using dye injections in 1956, Pressman et al[4] demonstrated horizontal lymphatic communications between the lateral paraglottic spaces via the preepiglottic space. In 1962, Tucker and Smith[5] used the whole-organ section technique for studying laryngeal cancer and in 1963 Tucker[6] showed residual cancer in the preepiglottic space after radiotherapy. Bocca et al[7] described the high incidence of preepiglottic space invasion in supraglottic cancer and stressed the importance of the preepiglottic space as a prognosticator of cervical metastasis. Using serial sections, Kirchner[8] and Kirchner and Som[9] found a high incidence of preepiglottic space invasion in epiglottic cancer. Also from serially sectioned specimens, McDonald et al[10] reported that 33 of 40 supraglottic cancers had preepiglottic space invasion. These tumors were confined to the supraglottis but were not exclusively epiglottic primaries. The anatomic definition and prognostic significance of the preepiglottic space were debated at the Centennial Conference on Laryngeal Cancer[11] in 1974. The consensus was that preepiglottic space invasion predisposed to cervical metastasis and a worse prognosis. Partly as a result, the staging system[12,13] was modified in 1977, with preepiglottic space invasion being designated T3. However, the endoscopist still faces the difficulty of assessing an invisible third dimension, invasion of the preepiglottic space, whether by inspection and palpation or by imaging methods.[11] Zeitels et al[14] studied 22 supraglottic cancer specimens originally staged T1 and T2 and found preepiglottic space invasion in 7. This invasion had not been identified by computed tomography.

In a series of 105 patients, Nadol[15] reported cervical metastasis in 3 of 19 (16%) suprahyoid epiglottic cancers and in 28 of 86 infrahyoid lesions (33%) based upon pretreatment clinical staging. In that series, he concluded that the increased incidence of cervical metastasis with infrahyoid lesions resulted in a poorer 5-year survival: 56% in infrahyoid lesions and 79% in suprahyoid.

MATERIALS AND METHODS

To determine the incidence of preepiglottic space invasion and cervical node metastasis in a series of T1 and T2 epiglottic lesions, a study of 36 specimens resected by total or partial laryngectomy was undertaken. Infrahyoid lesions (27) outnumbered suprahyoid lesions (9). Whole-organ sections were used to evaluate 26 laryngectomy specimens at the Yale–New Haven Medical Center, and 10 epiglottectomy specimens at the Boston Veterans Affairs Medical Center were studied by histopathology. Tumor volume was not quantified. Cervical lymph node metastasis was established in 14 cases either by neck dissection or by the appearance of metastatic disease in the cervical nodes during the follow-up period.

RESULTS

Six of the 12 T1 and 18 of the 24 T2 epiglottic le-

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sions had unrecognized preepiglottic space invasion (see Figure, A). None of the 9 suprahyoid lesions showed preepiglottic space invasion, whereas 24 of the 27 infrahyoid lesions showed some degree of invasion. Of the 3 infrahyoid cancers without preepiglottic space invasion, 1 was a verrucous carcinoma and another was a mucoepidermoid carcinoma located at the petiole. The third was a squamous cell carcinoma (see Figure, B).

Cervical metastasis occurred in 12 of 24 patients with preepiglottic space invasion. Two of the 24 had been treated by therapeutic neck dissections, and metastatic carcinoma was confirmed pathologically. Elective neck dissection was performed in 14 of the 24 patients with preepiglottic invasion. Seven of the 14 (50%) patients who underwent elective neck dissection had cervical metastasis on pathologic examination. Eight other patients with preepiglottic space invasion and clinically negative necks were treated with radiotherapy. In 3 of these patients, cervical node metastasis eventually developed.

Two of the nine patients with cancer in the suprahyoid epiglottis presented with cervical lymphadenopathy, and metastatic disease was confirmed at neck dissection in both cases. However, both surgical specimens revealed occult invasion of the tongue base. Two other patients with suprahyoid lesions and clinically negative necks underwent neck dissection. Neither had extension outside the larynx or cervical metastatic disease on pathologic examination. Of the remaining five undissected necks in patients with suprahyoid lesions without preepiglottic space invasion, three received radiotherapy to the cervical lymph nodes. None of the five patients has developed metastatic disease during a follow-up period of at least 3 years.

DISCUSSION

The natural fenestrae of the epiglottis below the level of the hyoepiglottic ligament predispose to early preepiglottic space invasion by anterior cancer penetration (see Figure, A).[1,6,8,10,14-17] Clinical assessment of the preepiglottic space is difficult for several reasons. Direct laryngoscopic view of the lower epiglottis is largely two-dimensional and tangential. Instrumental and digital palpation of the vallecula above the hyoepiglottic ligament (the roof of the preepiglottic space) is usually unrevealing unless there is extensive invasion of this space. Radiographic imaging does not provide sufficient resolution for identifying a small volume of tumor (see Figure, A).[14,15,18]

The data generated from this review justify a high level of suspicion that preepiglottic space invasion has occurred with infrahyoid cancer, even with those lesions in an early clinical stage. Twenty-four of the 27 infrahyoid T1 and T2 lesions (89%) had preepiglottic space invasion. However, of the 3 infrahyoid lesions without preepiglottic space invasion, 1 was a verrucous carcinoma and 1 was a mucoepidermoid carcinoma. All the other lesions were squamous cell carcinoma, and 24 of the 25 (96%) exhibited invasion of the preepiglottic space (see Figure, C) and had therefore been understaged. In the present study none of the 9 lesions of the suprahyoid epiglottis invaded the preepiglottic space.

The control rates for treating cancer of the infrahyoid epiglottis by radiotherapy have not equaled those for surgery.[19,20] This may be due in part to the poor blood supply in the preepiglottic tissue, and in part to the difficulty in clinical assessment after radiotherapy. Once the surface has healed, detection of recurrent or residual disease is often delayed, even with deep biopsies.[19]

The preepiglottic space contains no lymph nodes, but it does contain lymphatic channels that allow cervical metastasis.[1,3,4,21,22] The occurrence of cervical metastasis with preepiglottic space invasion has been previously noted by Ogura,[23] Bocca et al,[7] and DeSanto.[24]

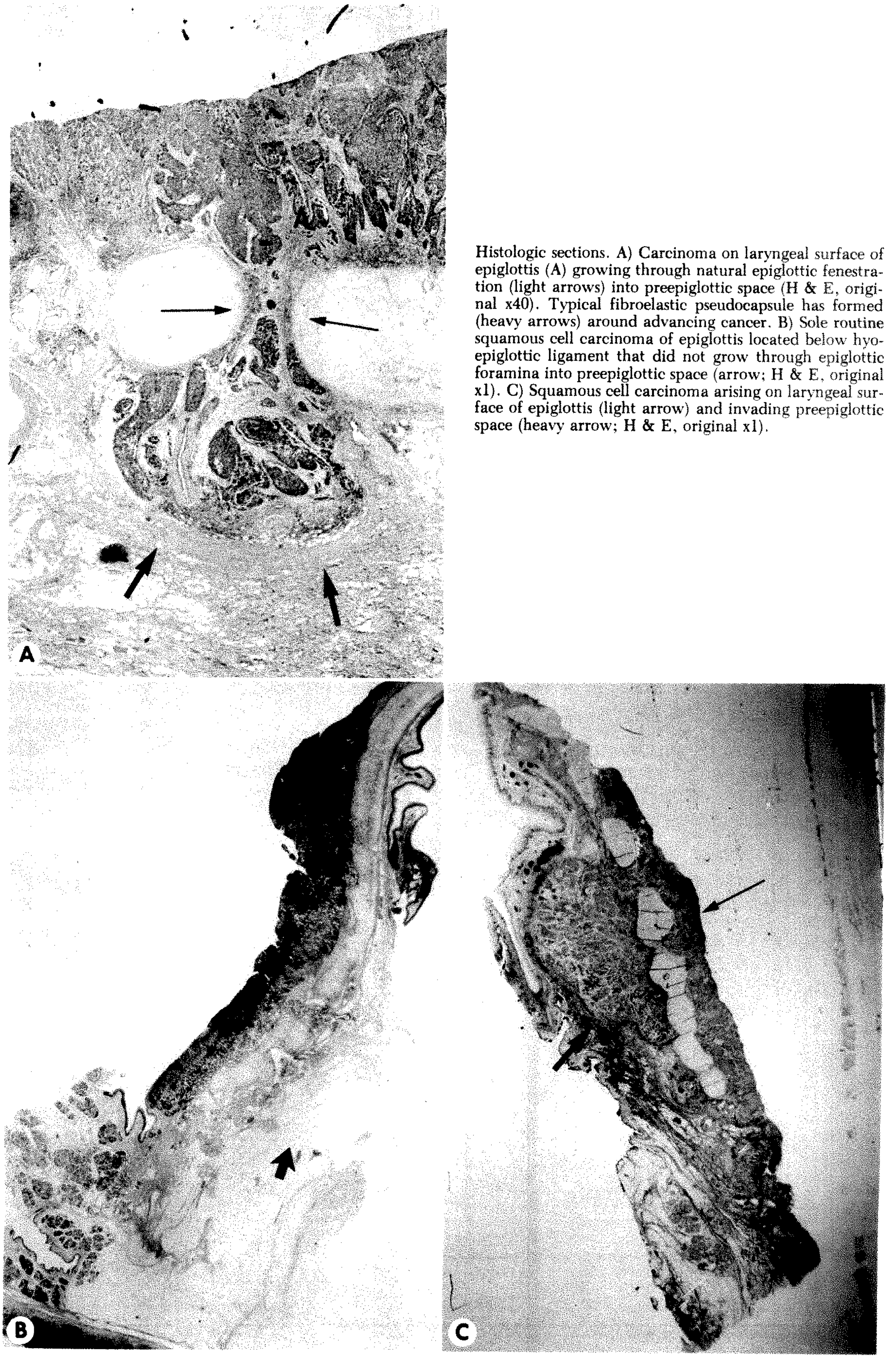
In the present study, 1 of the 10 patients with cancer confined to the epiglottic mucosa had metastasis to the neck. This patient had a high-grade mucoepidermoid carcinoma on the petiole. Cervical metastasis often occurred with preepiglottic space invasion (12 of 24) or with tongue base invasion (2 of 2). It is possible that some of the 5 patients with preepiglottic space invasion and clinically negative necks treated by full-course radiotherapy may have had occult cervical node metastasis, so that the incidence of cervical metastasis may be understated. Similarly, in the suprahyoid group there could have been occult cervical metastasis in those necks that were irradiated. Since the neck is usually the site of failure in supraglottic cancer,[11,24-29] aggressive regional management is prudent with any infrahyoid epiglottic carcinoma.

The present study does not determine whether the incidence of cervical metastasis depends on the volume of tumor within the preepiglottic space. For this reason, further study of this group of surgical specimens is being made in an effort to correlate tumor volume and metastatic rates.

CONCLUSIONS

1. Preepiglottic space invasion by squamous cell carcinoma involving the epiglottis inferior to the hyoepiglottic ligament is usual, even in those lesions without clinical evidence of invasion.

2. Invasion of the preepiglottic space by squamous cell carcinoma limited to the epiglottis above the hyoepiglottic ligament did not occur in any of the nine specimens in this study; this finding suggests that such invasion from T1 or T2 lesions rarely occurs.



Histologic sections. A) Carcinoma on laryngeal surface of epiglottis (A) growing through natural epiglottic fenestration (light arrows) into preepiglottic space (H & E, original x40). Typical fibroelastic pseudocapsule has formed (heavy arrows) around advancing cancer. B) Sole routine squamous cell carcinoma of epiglottis located below hyoepiglottic ligament that did not grow through epiglottic foramina into preepiglottic space (arrow; H & E, original x1). C) Squamous cell carcinoma arising on laryngeal surface of epiglottis (light arrow) and invading preepiglottic space (heavy arrow; H & E, original x1).

3. Cervical node metastasis occurred in half of the T1 and T2 epiglottic lesions lying below the hyoepiglottic ligament.

4. The relationship between tumor volume in the preepiglottic space and cervical metastasis is still to be determined.

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CARCINOMA IN SITU AND MICROINVASIVE SQUAMOUS CELL CARCINOMA OF THE VOCAL CORDS

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A retrospective study was undertaken to assess the outcome of 20 patients who underwent microlaryngoscopy and stripping of their vocal cord(s) with and without laser ablation of adjacent tissue for carcinoma in situ and/or microinvasive squamous cell carcinoma of the glottic larynx. Eighteen of 20 (90%) patients are currently free of disease following this course of treatment. The two treatment failures were in patients who had been lost to follow-up. A structured approach is presented to the clinical management of carcinoma in situ and microinvasive squamous cell carcinoma. We propose that the technique of tissue removal, microcup forceps stripping with or without laser ablation, is not the critical determinant of successful outcome, but rather that compulsive follow-up is most important to ultimate treatment success.

KEY WORDS — carcinoma in situ, squamous cell carcinoma, vocal cords.

INTRODUCTION

Carcinoma in situ and microinvasive squamous cell carcinoma (SCCA) of the vocal cords have generated much discussion in the last quarter century. As the biologic nature of these lesions has become better understood, their management has evolved. Yet, because these relatively infrequent lesions are initially treated successfully with a multitude of ablative techniques, including vocal cord stripping, laser excision, and radiotherapy, the best approach to their management is often not clear.

A retrospective study was undertaken to assess the outcome in 20 patients who underwent microlaryngoscopy and stripping of their vocal cord(s) with and without laser ablation of adjacent tissue for carcinoma in situ and/or microinvasive SCCA of the glottic larynx. On the basis of the experience at our institution with this spectrum of disease, we propose that the technique of tissue removal, microcup forceps stripping with or without laser ablation, is not the critical determinant of successful outcome, but rather that compulsive follow-up is most important to long-term treatment success. In addition, a structured approach is presented for the clinical management of carcinoma in situ and microinvasive SCCA.

PATIENTS, METHODS, AND MATERIALS

The medical charts of patients who had undergone microlaryngoscopy at the Eye and Ear Hospital of Pittsburgh between January 1, 1983, and December 31, 1987, were reviewed. The study population consisted of those patients with the diagnosis of carcinoma in situ or microinvasive SCCA of the glottis.

Carcinoma in situ was defined as the presence of full-thickness epithelial disarray and atypia associated with an intact basement membrane. Microinvasive SCCA was defined as full-thickness epithelial disarray and atypia associated with discrete foci of invasion through the basement membrane.

Standard suspension laryngoscopy was performed by using either Jako or Dedo wide-bore laryngoscopes. Microcup forceps were used to strip the mucosa off the vocal fold, leaving the underlying vocalis muscle undisturbed. If adjacent mucosa or underlying muscle was suspect for superficial invasion, the carbon dioxide laser was employed to vaporize these areas.

RESULTS

Two hundred fifty-seven patients were identified who had undergone microlaryngoscopy during the course of the study period. Diagnoses of glottic carcinoma in situ in 13 patients and glottic microinvasive SCCA in 7 patients were determined. Follow-up ranged from 27.3 months to 69.3 months, with an average of 46.9 months. Sixteen patients were men and 4 were women.

Eighteen of 20 patients (90%) were treated endoscopically with ultimate disease control. The laser was used in 12 of 20 patients. Two patients developed invasive SCCA, both of whom were lost to follow-up after biopsy-proven disease-free intervals of 16 and 28 months. One patient developed a contralateral T1 SCCA 64 months after his initial presentation with carcinoma in situ and was treated with radiotherapy. The other patient developed an ipsilateral T3 SCCA 65 months after his initial presentation with carcinoma in situ and was suc-

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REPRINTS — Eugene N. Myers, MD, Dept of Otolaryngology, Eye and Ear Institute, Suite 500, 203 Lothrop St, Pittsburgh, PA 15213.

cessfully salvaged with a total laryngectomy. The laser was not used in the treatment of either patient.

Recurrent carcinoma in situ or microinvasive SCCA developed in an additional four patients after a biopsy-proven minimum disease-free interval of 22 months. Two patients developed ipsilateral recurrence and two patients developed contralateral recurrence. The laser was used in the endoscopic treatment of all four patients. All four patients remain free of disease after further endoscopic treatment.

The time interval between the biopsy-proven absence of carcinoma in situ or microinvasive SCCA and the development of recurrent carcinoma in situ, microinvasive SCCA, or invasive SCCA ranged between 22 and 37 months, with an average of 29 months. The average number of procedures required by patients following the initial microlaryngoscopy was 2.25, with a range of 1 to 5. Nineteen of 20 patients were smokers, with an average tobacco-smoking history of 43.6 pack-years.

DISCUSSION

Great strides have been made in understanding the biology of carcinoma in situ and microinvasive SCCA over the last 20 years. Following the 1974 Centennial Conference on Laryngeal Cancer, a general consensus was agreed upon as to the basic histologic definitions of carcinoma in situ and microinvasive SCCA.[1] However, as Barnes[2] has noted, because of the marked subjectivity in interpreting vocal cord biopsies, the diagnosis is often not straightforward. This has led to incidences of isolated glottic carcinoma in situ and microinvasive SCCA that vary anywhere between 1% and 13% of all laryngeal carcinomas.[2] Our incidence of 6% (20 of 330) falls within the range of reported values.[3-6] As such, isolated glottic carcinoma in situ and microinvasive SCCA are relatively rare lesions.

With the recognition of carcinoma in situ and microinvasive SCCA as distinct entities along a spectrum of diseases, ranging between dysplasia and invasive SCCA, treatment programs have matured. Open neck procedures, and to a lesser extent radiotherapy, have largely given way to endoscopic techniques to control isolated glottic carcinoma in situ and microinvasive SCCA. The varied endoscopic methods have been embraced not only because they offer good control of disease while preserving voice, but because, just as important, they do not preclude further treatment options. As early as 1960, McCabe and Magielski[7] advocated the efficacy of limited laryngoscopic techniques. The studies of Fisher and Miller[8] and Miller[9] over a 25-year period also demonstrated that endoscopic procedures could be used with good success in isolated vocal cord carcinoma in situ. These studies also highlighted the potentially serious nature of the disease, as more than 10% of those patients treated endoscopically went on to develop invasive SCCA. Som[10] further elucidated the management of carcinoma in situ by using repeated vocal cord strippings successfully in 20 of 24 patients. The same technique was similarly employed by Bailey[11] with success in 22 of 25 patients with microinvasive SCCA. DeSanto,[3] while recognizing the utility of vocal cord stripping, pointed out that the term was often a misnomer, when in fact the procedure might also have included cordectomy, surgical diathermy, electrocoagulation, cryonecrosis, or even laser ablation. In recent years, the laser has been used with good success in these superficial vocal cord lesions.[3] Good control of disease has, in fact, been accomplished in almost all of the variations of endoscopic techniques, with perhaps the exception of simple biopsy.[12-20] Because of the relative rarity of isolated vocal cord carcinoma in situ and microinvasive SCCA, and because so many treatment modalities have proven successful, there has been no uniformity in treatment regimens. Ferlito et al[21] documented this lack of uniformity in an international survey of head and neck surgeons in 1981. It is interesting that in this survey the majority of respondents still treated isolated glottic carcinoma in situ with radiotherapy.

In the present study, control of disease was accomplished endoscopically in 18 of 20 patients. One patient with a T1 SCCA was salvaged with radiotherapy, and the other patient with a T3 SCCA was salvaged with a total laryngectomy. No differences were noted among those patients treated with the laser and stripping versus stripping alone, either in the recurrence of carcinoma in situ or microinvasive SCCA or in the development of invasive SCCA.

The concept of field disease is important in understanding vocal cord disease and the spectrum of disease ranging from dysplasia to carcinoma in situ or microinvasive SCCA to invasive SCCA. This progression generally represents the sequelae of long-term insults (usually tobacco smoke) to the vocal cord epithelium. Removal of a lesion does not guarantee that additional lesions will not appear at some later date on the same or opposite vocal cord, even if the inciting insult is removed. Similarly, whereas individual lesions are curable with radiotherapy, the irradiated field cannot be sterilized long-term from a tumor or dysplasia standpoint.[22] The only sure guarantee against further dysplasia and malignant change is organ removal, clearly not a treatment option. Many authors have demonstrated the development of second and third vocal cord carcinomas in situ, microinvasive SCCAs, and invasive SCCAs years after initial treatment with endoscopic or radiotherapeutic methods.[10,13,14,23] The present study confirms these observations, as 6 of 20 patients (30%) developed new vocal cord carcinoma in situ, microinvasive SCCA, or invasive SCCA after biopsy-proven disease-free intervals of

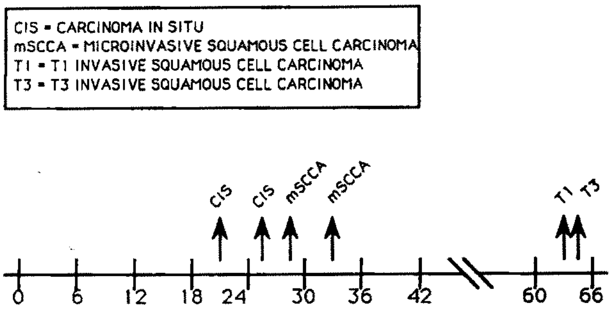

Fig 1. New-onset carcinoma in situ, microinvasive squamous cell carcinoma, and invasive squamous cell carcinoma.

at least 22 months (Fig 1). This potential for further dysplasia, even after definitive therapy, has been one of the strongest arguments against the use of radiotherapy for carcinoma in situ and microinvasive SCCA.

Recognition of the need for compulsive patient follow-up has been fundamental to the success of endoscopic techniques. The admonition of McGavran et al[24] that "therapeutic opportunism, social instability, doctor hopping and the patient's wish for an absolute, quick and guaranteed cure are inimical to the conservative management of [carcinoma in situ and] superficially invasive carcinoma of the vocal cord" remains true today. The present study powerfully demonstrates the need for long-term patient surveillance, as the only treatment failures were in the two patients who were lost to follow-up. These patients had shown no evidence of disease for 16 to 28 months, respectively, before being lost to follow-up, but developed invasive SCCA more than 5 years after their initial procedures. The four patients who had adequate follow-up and subsequently developed recurrent carcinoma in situ or microinvasive SCCA did so after disease-free intervals of at least 22 months and were all successfully managed with further endoscopic techniques (stripping with or without an adjunctive carbon dioxide laser).

Our treatment protocol for carcinoma in situ and microinvasive SCCA (Fig 2) is predicated upon close follow-up. Patients are strongly encouraged to abstain from smoking. Suspicious lesions noted at office examination are further investigated under microlaryngoscopy and stripped or submitted to biopsy as necessary. If a lesion does not strip well off the underlying vocalis muscle, the laser is often employed to ablate any suspicious adjacent or underlying tissue. Pathologic diagnosis of keratosis or dysplasia requires only follow-up examination in 3 months. If invasive SCCA is demonstrated, the patient is scheduled for conservation laryngeal surgery or radiotherapy. A diagnosis of carcinoma in situ or microinvasive SCCA requires repeat examination, microlaryngoscopy, and stripping in 6 weeks. Repeat stripping done prematurely can make pathologic examination considerably more difficult.[13] If no further carcinoma in situ or microinvasive SCCA is demonstrated in the pathologic specimen, the patient is examined again in 6 weeks. If this examination yields normal findings, the patient is seen in 3 months and then biannually thereafter if all examinations have normal findings.

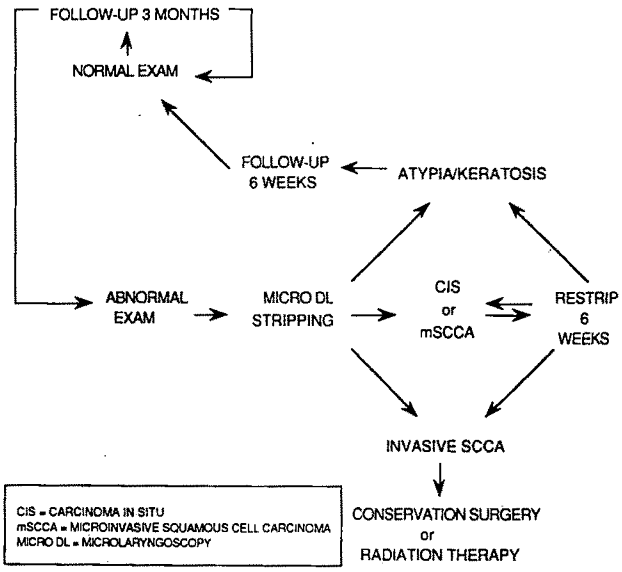

Fig 2. Management algorithm.

Relative contraindications to this approach include patients medically unable to undergo general anesthesia, patients unable to be followed up regularly, and patients in whom laryngeal examination, even under anesthesia, is not adequate. These subgroups of patients would be directed toward radiotherapy.

These data confirm multiple other reports that endoscopic excision of carcinoma in situ and microinvasive SCCA of the vocal cords is efficacious. It does not, however, demonstrate an advantage in use of the laser over microcup forceps stripping alone. Both techniques alone or in combination work well. Most important, the current data demonstrate that carcinoma in situ and microinvasive SCCA of the vocal cords represent a field disease with the potential for further dysplasia and metaplasia years after initial adequate treatment. Long-term patient surveillance is therefore essential.

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Ann Otol Rhinol Laryngol 100:1991

LARYNGEAL SYNKINESIS FOLLOWING REINNERVATION IN THE RAT

NEUROANATOMIC AND PHYSIOLOGIC STUDY USING RETROGRADE FLUORESCENT TRACERS AND ELECTROMYOGRAPHY

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The functional organization of laryngeal motoneurons in the nucleus ambiguus (NA) was evaluated in adult male rats before and after recurrent laryngeal nerve section and reinnervation. Using retrograde double labeling techniques with fluorescent probes, we obtained the number and position of labeled neurons by using the Bioquant 3-D imaging system. Reinnervation was documented by electromyography. In nine control animals vector analysis revealed significant ($p < .05$) separation of the posterior cricoarytenoid (PCA) muscle motoneurons and the thyroarytenoid and lateral cricoarytenoid (TA/LCA) muscle motoneurons. The PCA motoneurons were positioned ventromedially in the NA, and TA/LCA motoneurons were found dorsolaterally in the NA. Rostral-caudal separation was not significant. Electromyography revealed phasic electrical activity synchronous with respiration in the PCA, and activity synchronous with deglutition in the TA/LCA. In four animals surviving 15 weeks following recurrent laryngeal nerve section and primary neurorrhaphy, functional organization within the NA was lost and phasic motor unit activity synchronous with respiration was seen in the TA/LCA muscle as well as the PCA. Vector analysis revealed the reinnervating motoneurons for both the PCA and TA/LCA to be positioned dorsolaterally, similar to the control group TA/LCA motoneurons. These findings demonstrate a shift in the topographic organization of laryngeal motoneurons within the NA following reinnervation, with random organization occurring at the neurorrhaphy site.

KEY WORDS — electromyography, laryngeal paralysis, laryngeal synkinesis, motoneuron, neurorrhaphy, nucleus ambiguus, recurrent laryngeal nerve reinnervation.

INTRODUCTION

Recurrent laryngeal nerve (RLN) injury, traumatic or idiopathic, and subsequent reinnervation of the paralyzed larynx frequently results in synkinesis. Synkinesis may be defined as the synchronous contraction of muscles that normally do not contract together. Within the larynx this refers to the simultaneous contraction of antagonistic muscle groups, with the end result being an immobile vocal cord fixed by opposing forces (Fig 1A). Inappropriate innervation of the stronger adductor muscles (the thyroarytenoid [TA], interarytenoid [IA], and lateral cricoarytenoid [LCA] muscles) counteracts the weaker forces applied by the abductor (the posterior cricoarytenoid [PCA]) muscle and results in medialization of the vocal cord. This condition is, in part, responsible for the airway compromise occurring after bilateral vocal cord paralysis in humans, as well as the failure to achieve functional improvement following reinnervation procedures after transection of the RLN.[1] The overall incidence of synkinesis following experimental laryngeal reinnervation varies from 66% to 88%, which is believed to be comparable to the incidence in humans.[2-5]

Laryngeal synkinesis is hypothesized to occur as the result of misdirected axonal regeneration and subsequent inappropriate reinnervation of abductor laryngeal muscles by adductor fibers and vice versa.[6] Axons innervating both abductors and adductors are normally present in the RLN. Evidence of inappropriate reinnervation was reported by Siribodhi et al,[2] who first proposed the mechanism of aberrant regeneration of nerve fibers. By means of electromyography (EMG) following regeneration of repaired RLNs, inappropriate EMG activity with no meaningful laryngeal function was documented in three of four surviving dogs. Other reports confirm Siribodhi's original findings,[3,7-9] although a few studies report return of adductor function following reinnervation.[10-12] The return of adductor function may be the result of cricothyroid (CT) muscle activity, as suggested by Rice.[13]

Previous studies in rats,[14] rabbits,[15] cats,[16] and monkeys,[17] using horseradish peroxidase (HRP) retrograde labeling, have localized the somata of motoneurons innervating laryngeal muscles to the ipsilateral nucleus ambiguus (NA), the retrofacial nucleus, and the dorsal motor nucleus of cranial nerve X. The RLN axons arise from the motoneuron cell bodies in the NA. The NA maintains a narrow posi-

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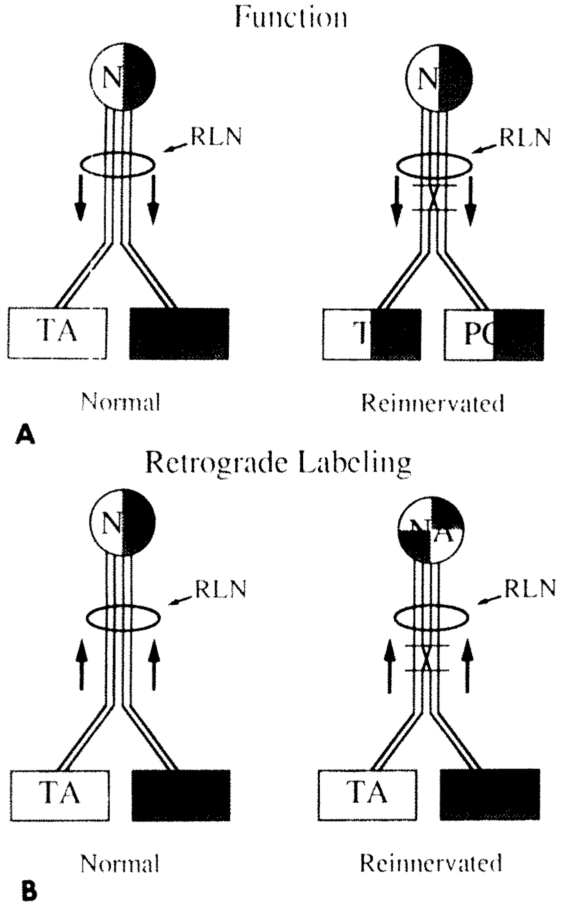

Fig 1. Functional organization of laryngeal motoneurons. RLN — recurrent laryngeal nerve. A) Diagram depicting somatotopic organization of motor units in normal animals and random reorganization at site of injury resulting in synkinesis. Shaded areas correspond to muscle with activity synchronous with respiration. Following reinnervation, activity synchronous with respiration is seen in both posterior cricoarytenoid (PCA) and thyroarytenoid (TA) muscles. B) Diagram depicting retrograde fluorescent labeling of nucleus ambiguus (NA) motoneurons in normal and reinnervated animals. Shaded areas correspond to biologic probe (fluorescent dye) used for retrograde labeling. On left, retrograde labeling of normal NA demonstrating two discrete populations of adductor (TA) and abductor (PCA) motoneurons. On right, retrograde labeling of antagonistic muscles following reinnervation would result in loss of separation of adductor and abductor motoneuron pools as result of random or nonselective reinnervation.

tion in the brain stem at the level of the upper medulla and lower pons, and lies adjacent to the ventral respiratory group motoneurons in the lateral tegmental region in the brain stem. The ventral respiratory group contains motoneurons innervating the diaphragm and accessory respiratory muscles, and appears to provide respiratory input to the laryngeal motoneurons.[18] Although slight variation occurs between different species, the cell bodies of motoneurons innervating the CT muscle in monkeys are found in the more rostral half of the NA dorsomedially, as well as in the adjacent retrofacial nucleus. The cell bodies of the motoneurons innervating the PCA, TA, LCA, and IA muscles are seen in the caudal half of the NA. The cell bodies of the PCA neurons are localized medially, the TA group are localized laterally, and the LCA and IA are sandwiched in between. These findings were confirmed in dogs[19] and cats[20] by using a double labeling method with retrograde fluorescent tracers. With this technique, Hisa and Sato[19] found little or no regional overlap in the motoneuron pools, although double-labeled cells innervating two synergistic muscles — the TA and the LCA — were identified.

Retrograde HRP has recently been applied to reinnervated laryngeal muscle groups. Using a freeze injury method to the RLN, Nahm et al[21] evaluated the effects of reinnervation on PCA motoneurons in the NA. The PCA cells in the normal animal were localized within the middle one third of the NA. Following reinnervation, PCA motoneurons were found in diminished numbers throughout the entire neuronal distribution of the RLN in the NA, suggesting random or nonselective reinnervation. Organization in the transverse plane was not evaluated.

Nonselective reinnervation has been documented in the hypoglossal nerve by using retrograde HRP labeling in cats,[22] and in the sciatic nerve in rats by using topographic nerve stimulation.[23] Other studies, however, have demonstrated evidence of selective reinnervation. Aldskogius and Thomander[24] reported selective reinnervation following facial nerve transection in neonatal rats and nonselective reinnervation in adult rats. Laskowski and Sanes[25] found selective reinnervation occurring with the serratus anterior and diaphragm muscles in neonatal rats and to a lesser degree in adult rats. The effects of age on selective reinnervation have not been investigated in the larynx.

The purpose of the present study is to assess the neuroanatomic changes and adaptive processes that result in laryngeal synkinesis following reinnervation after nerve section and repair. This is accomplished by placing two different fluorescent dyes, fast blue (FB) and diamidino yellow (DY), into the adductor (TA/LCA) and abductor (PCA) muscles and examining the three-dimensional (3-D) organization of labeled motoneurons in the NA of normal animals compared to reinnervated animals. If reinnervation occurs randomly at the anastomotic site, as is generally accepted, then retrograde labeling will result in loss of functional organization of laryngeal motoneurons in the NA (Fig 1B). However, if a selective process exists (ie, if one cell type has a selective advantage during reinnervation), a shift in position of motoneuron populations would be expected to occur regardless of the reinnervation process at the anastomotic site. The change in position and number of labeled nuclei will also be correlated with physiologic changes as assessed by EMG and fiberoptic laryngoscopy.

MATERIALS AND METHODS

Operative Technique. Adult male Sprague-Dawley rats (400 to 500 g) were anesthetized by intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). By means of a standard operating microscope, the larynx was approached through a midline incision. The TA and LCA muscles were treated together as the adductor group (TA/LCA) because of their small size and inability to be labeled separately (rats do not have an IA muscle). A window made in the anterior thyroid cartilage was used to expose the TA/LCA muscle. The PCA muscle was accessed by developing the tissue plane between the larynx and the esophagus, followed by the lateral rotation of the larynx.

To label the muscle fibers, HRP (type IV), FB, or DY (all Sigma, St Louis, Mo) crystals were inserted into the muscle with a 30-gauge needle. Excess dye was swabbed away following insertion of crystals to decrease contamination of other structures. For whole nerve labeling the RLN was sectioned approximately 0.5 cm caudal to its insertion into the laryngeal muscles and a 50% solution of HRP (distilled H_2O with 2% dimethyl sulfoxide) or FB crystals was placed into a 3-mm piece of Silastic tubing (diameter, 0.50 mm). The tubing was closed at one end and the proximal end of the sectioned nerve was placed into the other end of the tube and secured in position with 10-0 nylon suture.

The ipsilateral superior laryngeal nerve (SLN) was cut to minimize potential contamination from the CT muscle. When used, the cartilage window was closed with Gelfoam, and the neck was closed in layers. In preliminary studies, 3-day survival periods were found to be optimal for retrograde labeling in both the HRP and fluorescent dye methods.

Electromyography. Electromyograms were obtained prior to RLN section and 15 weeks following neurorrhaphy to document reinnervation of the muscle groups. Sectioning the ipsilateral SLN eliminates potential distant electrical activity from the CT muscle. Following anesthesia and exposure of the laryngeal muscles, a monopolar electrode was placed in the right PCA muscle and the right TA/LCA muscle. In addition, for each monopolar electrode, a reference electrode and a ground electrode were placed subcutaneously. The EMG signals were amplified by a Data Inc, 2124 model 2 instrumentation amplifier and stored in a Tektronix 2232 digital storage oscilloscope. Standardized amplification (gain, 100; high frequency filter, 10,000 Hz; low frequency filter, 30 Hz) and recording levels were used.

Horseradish Peroxidase Labeling. Anesthetized animals (intraperitoneal Equithesin 3 μL/g) were perfused intracardially with 4% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.4. Brain stems were carefully removed from the skull and postfixed for 12 hours in fixative with 10% sucrose followed by 12 hours in fixative with 30% sucrose. The brain stems were frozen to −70°C and sectioned in the transverse plane at 40 μm by means of a sliding microtome. Sections were processed according to the techniques described by Mesulam et al.[26,27] The HRP-labeled tissue sections were visualized with brightfield microscopy.

Fluorescent Dye Labeling. Anesthetized animals were perfused intracardially with 4% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4. Brain stems were carefully removed from the skull and postfixed for 12 hours in fixative with 10% sucrose followed by 12 hours in fixative with 30% sucrose. Brain stems were frozen to −70°C and sectioned at 25 μm in the transverse plane on a Reichert-Jung 2800 Frigocut N cryostat. Sections were transferred to glass slides, dehydrated in a graded series of ethanol, cleared in xylene, and coverslipped with DPX mountant prior to viewing. Fluorescent-labeled neurons were visualized with a Leitz Aristoplan microscope. A Leitz D fluorescent cube (excitation wavelength 355 to 425 μm) was used for fluorescence activation for both FB and DY.

EXPERIMENTAL DESIGN

Animals were divided into five groups. Groups 1 through 4 were control animals to determine the normal distribution of PCA and TA motoneurons, and the degree of contamination from surrounding extralaryngeal muscles. Group 5 consisted of animals undergoing reinnervation for comparison to control animals.

In control group 1, the whole RLN was labeled either with HRP (n = 6) or FB (n = 3). These controls determined the location of RLN cell bodies in the NA. The rostral-caudal range of whole RLN labeling represented the limits of neuronal labeling for the PCA and TA/LCA motoneurons in the NA.

In control group 2, PCA and TA/LCA muscles were labeled with intramuscular crystal insertion following division of the SLN, thus eliminating possible CT motoneuron labeling. Retrograde labeling utilized either HRP (n = 6) inserted into the PCA and contralateral TA/LCA or dual fluorescent dye labeling (n = 9). Fast blue was used to label the PCA in seven animals and TA/LCA in two animals, while DY was used to label the ipsilateral antagonist PCA (n = 2) or TA/LCA (n = 7) muscle. The nine fluorescent-labeled animals in control group 2 were compared to the reinnervated animal group.

Control group 3 (n = 3) determined the extent of extralaryngeal muscle contamination following TA/LCA and PCA muscle labeling. Fast blue and DY were inserted intramuscularly into the PCA and TA/LCA muscles, respectively, followed by imme-

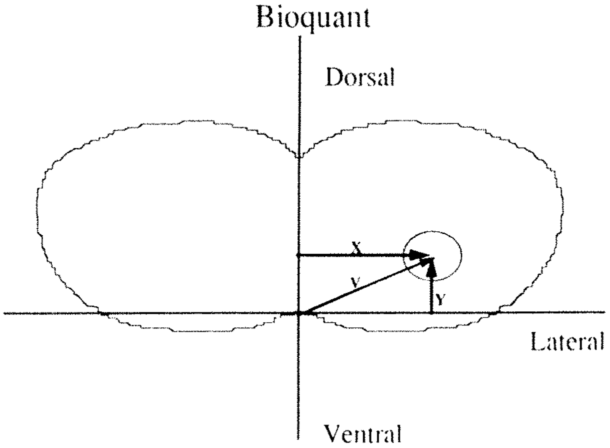

Fig 2. Model depicting transverse brain stem section and method for assigning x, y coordinates for each labeled motoneuron relative to ventral midline reference point of each transverse section using Bioquant 3-D reconstruction program. x — medial-lateral dimension, y — dorsal-ventral dimension, v — vector = $\sqrt{x^2 + y^2}$.

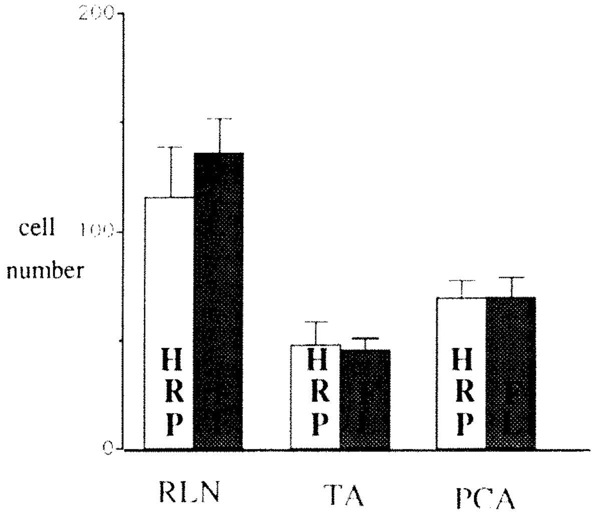

Fig 3. Comparison between horseradish peroxidase (HRP) and fluorescent dye (FL) neuronal labeling demonstrated no statistical advantage of either method when labeling whole recurrent laryngeal nerve (RLN; control group 1) or specific muscles (control group 2). Less uptake occurs with intramuscular injections when compared to whole-nerve labeling. Error bars — standard error of mean.

diate sectioning of the RLN and SLN. Neuronal labeling in this group should represent contamination of the surrounding pharyngeal and strap muscles and not the denervated CT, PCA, or TA/LCA musculature.

In control group 4 (n = 2), CT and extralaryngeal contamination during labeling of TA/LCA and PCA motoneurons was determined by labeling with the SLN (and external branch to the CT) intact. The RLN was divided. Differences observed between group 3 and group 4 represent the contribution of CT contamination.

In six animals, experimental group 5, the right RLN was sectioned approximately 0.5 cm proximal to the site of insertion into the larynx. The nerve was then immediately sutured together with 10-0 nylon suture. Laryngoscopy with a fiberoptic pediatric bronchoscope was used to document immobility of the right vocal cord. The surgical site was closed in layers and the animals were kept alive for 15 weeks to allow axonal regeneration. Fluorescent labeling was performed as described for control group 2. The PCA muscle was labeled with FB and the TA/LCA muscle was labeled with DY. Retrograde labeling was assessed after a 3-day survival period.

ANALYSIS OF DATA

The number and position of fluorescent-labeled neurons were obtained by using the Bioquant 3-D imaging system from R&M Biometrics Inc (Nashville, Tenn). Three-dimensional reconstructions of the brain stems were created by using the Bioquant system. The x and y coordinates and resultant vectors were determined for the FB- or DY-labeled neurons in each transverse section by using the ventral midline of each section as the origin or reference point (Fig 2). Rostral-caudal locations of labeled neurons were measured in the z axis relative to the obex. To eliminate double counting of neurons, absolute cell counts were adjusted by using the total count method defined by Abercrombie[28] for both HRP- and fluorescent dye–labeled brain stems.

Differential analyses of the PCA and TA/LCA neuronal population locations for each animal were performed by comparing the x, y, and vector values for the FB- and DY-labeled motoneurons. Two-tailed unpaired *t* tests on each normal animal and each reinnervated animal determined if statistically significant separation of neuronal populations existed.

To compare the position of PCA and TA/LCA neurons in the normal group to that in the reinnervated group, two-way mixed analysis of variance (ANOVA) was performed by using the mean x, y, and vector values for each animal. Separate ANOVAs were performed for each dimension. Posthoc specific comparison between these mean positions were made by using the Newman-Keuls test.

RESULTS

Labeling. To determine quantitatively the ability of FB and DY to label laryngeal motoneurons, comparisons between the HRP and fluorescent dye retrograde labeling were performed. When comparing absolute numbers of labeled soma, no statistically significant advantage to either biologic probe was observed, regardless of whether whole-nerve or intramuscular labeling was performed (Fig 3). Therefore, all further studies used FB and DY.

Labeling of the whole RLN (control group 1) demonstrated laryngeal motoneurons ranging from 1.6 mm below the obex to approximately 0.6 mm above the obex, with a few scattered cells more

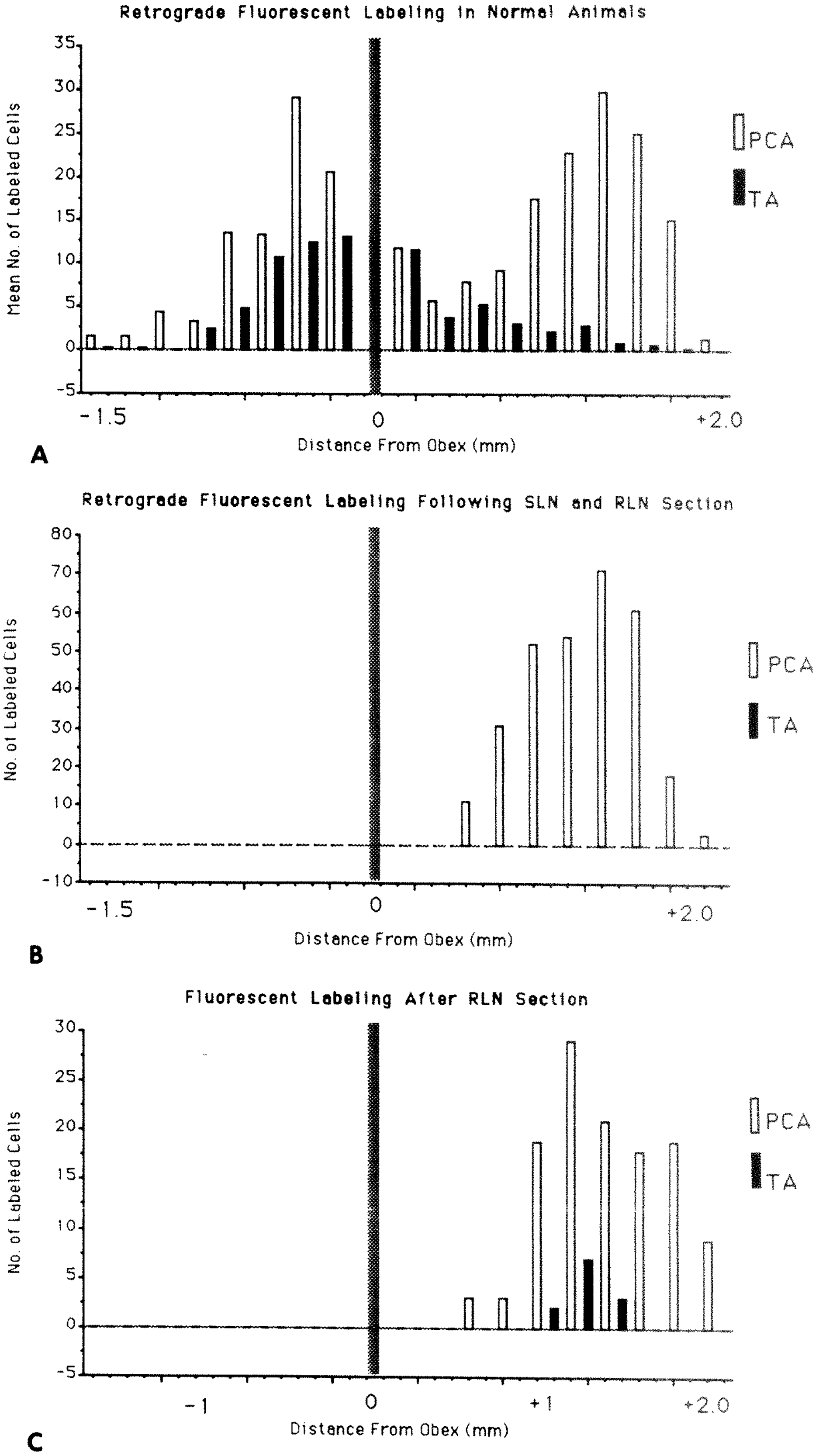

Fig 4. Rostral-caudal labeling of laryngeal motoneurons in control groups. A) Histogram showing mean number of cells labeled (n = 6) above and below obex (0), following double fluorescent labeling of PCA (fast blue) and TA (diamidino yellow) in normal rat. Separation of cells in rostral-caudal dimension does not occur. Large population of cells labeled with fast blue rostral to obex (0.6 to 2.0 mm) represents hypopharyngeal contamination (see B and C). B) Histogram showing number of cells labeled above and below obex (0), following double fluorescent labeling of PCA (fast blue) and TA (diamidino yellow) of single rat after transection of recurrent laryngeal nerve (RLN) and superior laryngeal nerve (SLN). Population of cells rostral to obex labeled with fast blue represents contamination of hypopharyngeal muscles. C) Histogram showing mean number of cells labeled (n = 3) above and below obex (0), following double fluorescent labeling of PCA (fast blue) and TA (diamidino yellow) after transection of RLN only. Labeling with diamidino yellow is seen in region above obex in addition to fast blue labeling (hypopharyngeal contamination), and results from contamination of cricothyroid muscle (innervated by SLN) during TA muscle labeling.

rostral (Fig 4A). Intramuscular injections into the PCA and TA/LCA with SLN sectioning (control group 2) resulted in labeling from 1.6 mm below the obex to 2.0 mm above the obex (Fig 4B). The more rostral labeling in control group 2 compared to whole RLN labeling (0.6 to 2.0 mm) resulted from extralaryngeal muscle contamination as demonstrated by control groups 3 (Fig 4B,C) and 4. Fol-

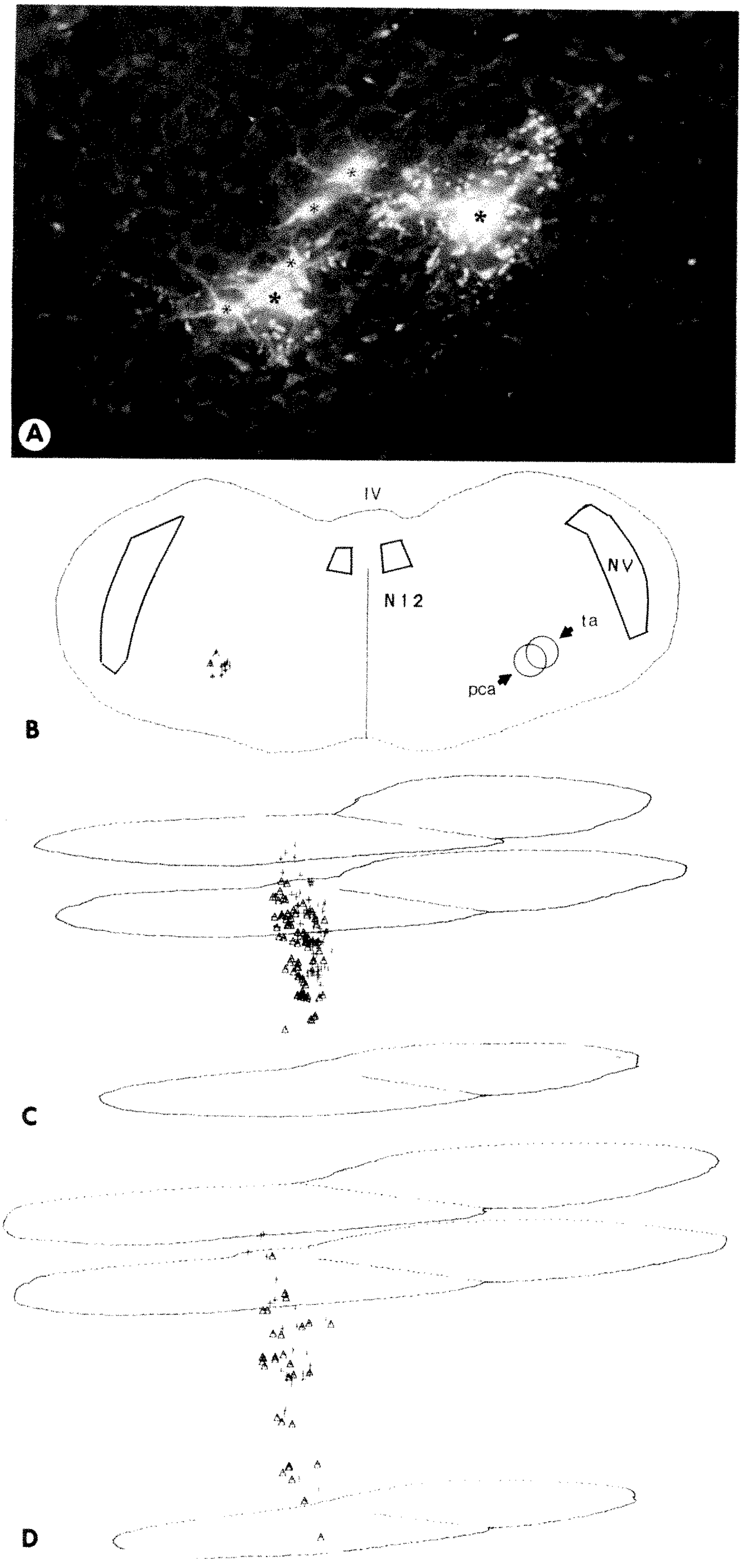

Fig 5. Nucleus ambiguus following retrograde labeling of motoneurons. A) Photomicrograph of transverse section through NA of normal rat following double labeling with fluorescent dyes. Diamidino yellow labels nucleus in TA/LCA (adductor) neurons (bold asterisks) bright yellow with some dye contamination found in cytoplasm and surrounding cells. Fast blue labels cytoplasm of PCA (abductor) motoneurons (light asterisks) with no spread into surrounding structures. Distinct separation of populations can be discriminated. B-D) △ — TA/LCA motoneurons, + — PCA motoneurons. B) Computer-generated reconstruction of transverse section of normal brain stem at level of obex showing location of TA/LCA and PCA motoneurons on left, and schematic illustration of two distinct motoneuron pools on right. IV — fourth ventricle, N12 — nucleus of cranial nerve XII, NV — nucleus of cranial nerve V. C) Three-dimensional reconstruction of control animal in which upper section is 600 μm rostral to obex, and lowest section is 1,600 μm caudal to obex. Intermediate section is at level of obex. Overlapping but distinct TA/LCA and PCA motoneuron pools are seen. D) Three-dimensional reconstruction of reinnervated animal. Uppermost section is 600 μm rostral to obex, and lowest section is 1,600 μm caudal to obex. Intermediate section is at level of obex.

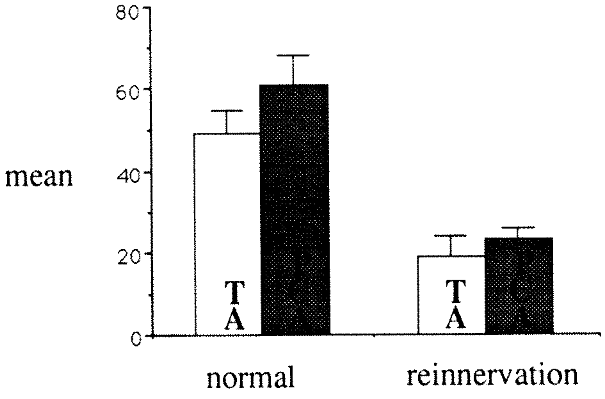

Fig 6. Absolute labeled cell count for normal (n = 9) and reinnervated (n = 4) animals.

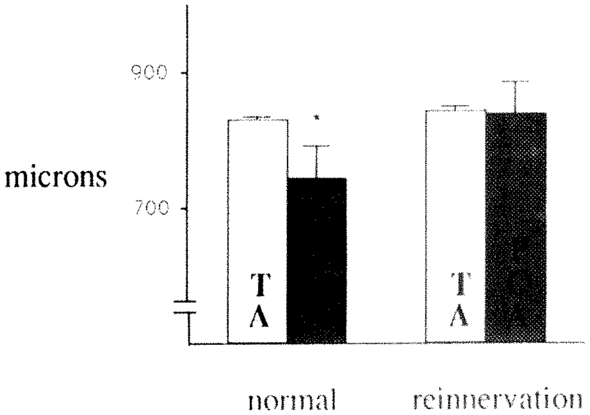

Fig 7. Mean y coordinate values are shown for TA and PCA muscles in normal animals (n = 9) compared to TA and PCA muscles in reinnervated animals (n = 4). Y coordinate value corresponds to dorsal-ventral position in transverse brain stem section relative to ventral midline reference point (see Fig 2). Asterisk — significant difference ($p < .05$) from three comparison groups.

lowing RLN plus SLN sectioning (control group 3) and RLN sectioning alone (control group 4), labeled neurons were found 0.6 mm rostral to the obex in the NA, and also in the retrofacial nucleus and the dorsal motor nucleus of the vagus (0.6 to 2.0 mm above the obex). These labeled somata are attributed to SLN contamination during TA/LCA injections by way of the CT, and vagal contamination via innervation of extralaryngeal muscles during PCA injection. Fast blue labeling of the CT motoneurons via the SLN (control group 4) demonstrated fluorescent motoneurons more than 0.6 mm above the obex. Less than 3% of the CT and extralaryngeal muscle motoneurons were seen caudal to the upper margin of the RLN range. From these parameters, only cells labeled within the range of the RLN (1.6 mm below the obex to 0.6 mm rostral to the obex) were considered PCA or TA/LCA motoneurons in control and reinnervated animals.

Normal Controls. Using the Bioquant 3-D program, two-dimensional reconstructions were made from each transverse section in the x-y plane for the normal controls (Fig 5A,B). The adductor neurons (TA/LCA) were located more dorsal and lateral than the abductor neurons (PCA). Three-dimensional reconstructions revealed distinct overlapping columns of TA/LCA and PCA motoneurons in all nine normal rats (Fig 5C). The labeled TA/LCA somata formed a column more dorsal and lateral than the PCA somata. In the rostral-caudal dimension, there was no pattern of separation between the abductor and adductor motoneurons. Neuronal labeling was found only ipsilateral to the labeled muscle. Double labeling of cells distinguished by fluorescent yellow nuclei and fluorescent blue cytoplasm (neurons innervating both muscles) ranged from 0% to 8% of the total number of labeled cells in nine normal animals.

Statistical analysis of the motoneurons labeled from PCA and TA/LCA muscles of normal rats revealed the TA/LCA neurons to be significantly more lateral than the PCA neurons in all nine animals ($p < .05$). The TA/LCA neurons were also found to be significantly more dorsal than the PCA neurons in eight of nine animals ($p < .05$). Vector analysis was also significant for separation of the TA/LCA and the PCA neuron populations in all nine animals ($p < .05$). In three of the nine normal animals, the PCA neurons were significantly more caudal than the TA/LCA. The converse was true for three other normal animals, with no statistically significant separation in the remaining three animals.

Experimental Group 5. Six animals underwent RLN section and primary repair. Two of these animals died during the axonal regeneration period. Of the remaining four animals, two observations were made following 3-D reconstructions. First, the PCA and TA/LCA labeled neurons were no longer spatially segregated, but instead were widely spread throughout the NA with no statistically significant separation of the PCA and TA/LCA columns in the x, y, or z axis (Fig 5D). Second, there was a 50% decrease in labeling of neurons in the NA following reinnervation (Fig 6). The loss of separation of PCA and TA/LCA neurons and the decreased labeling were found in all four animals. As in the normal animals, the neuronal labeling was found only ipsilateral to the labeled muscles. Double-labeled cells comprised 0% to 15% of all labeled motoneurons in the reinnervated animals.

Analysis of Normal Controls Versus Reinnervated Animals. When comparing the normal controls versus reinnervated animals, analysis of mean y coordinate values (dorsal-ventral dimension) in the transverse plane demonstrated significant separation between the dorsally located TA/LCA motoneurons and the ventrally located PCA motoneurons in normal animals, with loss of this separation in the reinnervated group (Fig 7). Reinnervating motoneurons for both the PCA and TA/LCA mus-

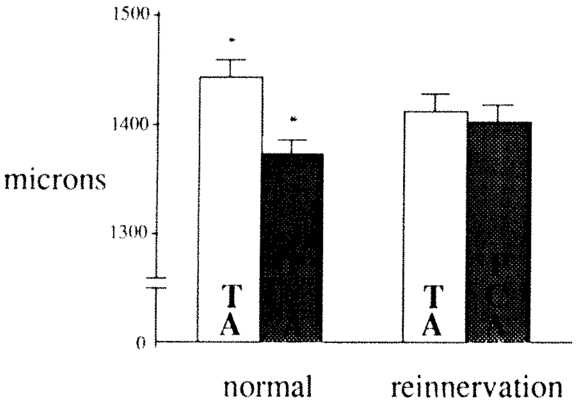

Fig 8. Mean x coordinate values are shown for TA and PCA muscles in normal animals (n = 9) compared to TA and PCA muscles in reinnervated animals (n = 4). X coordinate value corresponds to medial-lateral position in transverse brain stem section relative to ventral midline reference point (see Fig 2). Asterisk — significant difference (p < .05) from three comparison groups.

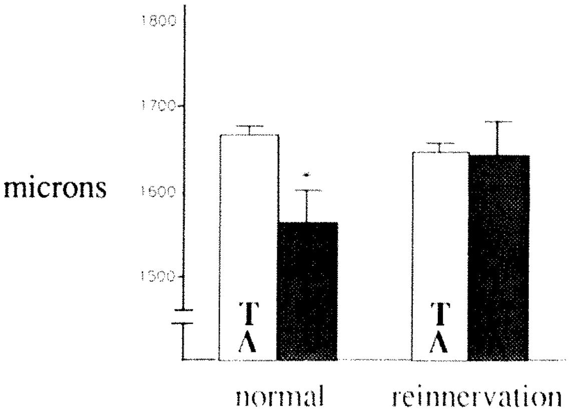

Fig 9. Mean vector values are shown for TA and PCA muscles in normal animals (n = 9) compared to TA and PCA muscles in reinnervated animals (n = 4). Resultant vector is obtained from x, y coordinates (see Fig 2) and represents distance from ventral reference point. Asterisk — significant difference (p < .05) from three comparison groups.

cles, however, tended to be found in a more dorsal position (greater mean y value), similar to normal TA/LCA motoneurons. Analysis of the mean x coordinate values for the medial-lateral dimension also showed significant separation of the PCA and TA/LCA motoneuron pools in the normal rats, with TA/LCA neurons positioned more laterally than the PCA motoneurons. Loss of separation occurred following reinnervation in the medial-lateral dimension, and motoneurons of both the PCA and TA/LCA muscles were found in a more lateral position (greater x value), similar to TA/LCA motoneurons in normal animals (Fig 8). Vector analysis demonstrated the mean vector for the TA/LCA motoneurons to be significantly greater than the mean PCA vector in normal controls. The difference between PCA and TA/LCA vector lengths was lost following reinnervation, and values for both the PCA and the TA/LCA muscles approached the length of the normal TA/LCA vector (Fig 9).

Group-by–muscle type mixed ANOVAs showed similar results in all three dimensions in the transverse plane. In all cases, there was no reliable main effect of group ($p > .05$), but there was a reliable effect of muscle type ($F_{1,11} = 27.8$, 50.6, 83.8 for the x, y, and vector measures, respectively, $p < .05$) and a reliable interaction ($F_{1,11} = 10.6$, 15.2, 33.0 for the x, y, and vector measures, respectively, $p < .05$). Post-hoc Newman-Keuls testing demonstrated, for each dimension, the position of PCA motoneurons in the normal animals to be reliably different ($p < .05$) from that of the other three groups of motoneurons (normal TA/LCA, reinnervated PCA, and TA/LCA).

In comparing the rostral-caudal positions, no separation was seen between the TA/LCA motoneurons and the PCA motoneurons in either the normal group or the reinnervated group. However, reinnervating neurons in general were found to be more caudal than the normal population (Fig 10). An overall effect of group ($F_{1,11} = 7.3$, $p < .05$) was seen in the rostral-caudal dimension, but no reliable effect of muscle type ($F_{1,11} = 0.3$, $p = .60$) or reliable interaction ($F_{1,11} = 1.1$, $p = .33$) was found.

Electromyography. In nine control animals EMG revealed phasic electrical activity synchronous with respiration in the PCA, and activity synchronous with deglutition in the TA/LCA. In the four animals surviving 15 weeks following RLN section and primary neurorrhaphy, phasic motor activity synchronous with respiration was seen in the TA/LCA muscle as well as the PCA (Fig 11).

DISCUSSION

The somatotopic arrangement of labeled neurons in this study, found in the lower two thirds of the NA in control animals, is consistent with previous work,[14,20] with a few exceptions. Separation of abductors and adductors was observed in the transverse dimension, although not in the rostral-caudal aspect as demonstrated by Portillo and Pasaro.[20] Portillo and Pasaro also found the PCA column to stretch further rostrally and mix extensively with the CT motoneurons — a finding not observed in our study. This difference may be due in part to our interpretation of what is a labeled laryngeal motoneuron and what is contamination. The range of laryngeal motoneuron labeling was defined in our study as the range of the whole RLN (control group 1), and only a very small rostral-caudal overlap occurred between the CT (control group 4) and the PCA and TA/LCA motoneuron populations. Except for labeling that was attributed to contamination from spread of dyes (control groups 3 and 4), we found no laryngeal motoneurons located outside the NA in the retrofacial nucleus or the dorsal motor nucleus of the vagus.

Double-labeled motoneurons (yellow nucleus sur-

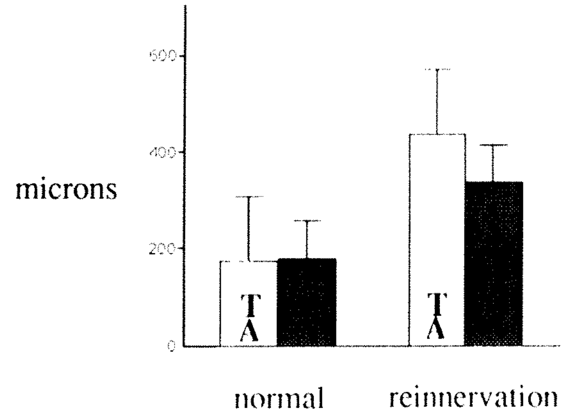

Fig 10. Mean z coordinate values are shown for TA and PCA muscles in normal animals (n = 9) compared to TA and PCA muscles in reinnervated animals (n = 4). Z coordinate value corresponds to rostral-caudal position in 3-D reconstruction relative to obex. By convention, positive values indicate distance caudal to obex. While there is more caudal shift following reinnervation, no significance was demonstrated.

rounded by blue cytoplasm) were found consistently in both normal and reinnervated populations. Previously, Hisa and Sato[19] found double-labeled motoneurons from both the TA and the LCA, muscles of similar function. Dual-functioning neurons were suggested to be involved in coordination of phonation and swallowing.[19] One might expect to see more of these neurons following a period of random axonal regeneration, but this was not observed in the present study. No new conclusions can be drawn at this time concerning the significance of these cells.

The loss of functional organization in the NA in the reinnervated animals supports the findings of Nahm et al.[21] Our study further defines the neuroanatomic changes that occur with reinnervation of the larynx. Reinnervating neurons for both the PCA and TA/LCA muscles tended to be located within a similar position in the brain stem as the TA/LCA motoneurons in normal animals.

Positional shifts seen in the transverse plane may occur as the result of selective reinnervation by the TA/LCA neurons or a subpopulation of the more dorsal-lateral motoneurons. This may be explained by metabolic or enzymatic differences between the abductor and adductor populations contributing greater regenerative capacity to the TA/LCA motoneurons. No previous documentation of selective reinnervation in the larynx has been reported. Previous studies in rats have shown a tendency for axial muscles to be reinnervated by their appropriate neurons. Selective reinnervation in this model was age-related, with younger animals showing greater selectivity.[25,29,30] Age-related selective reinnervation has also been demonstrated following facial nerve regeneration in the rat.[24]

A second possible explanation for the positional shift of reinnervating motoneurons is cell death of a specific motoneuron population. With relatively fewer PCA motoneurons surviving, the total population of reinnervating motoneurons would occupy a more dorsal-lateral position in the brain stem. Degeneration of motoneurons is supported by the decreased labeling of neurons documented in our study. Nahm et al[21] found a similar decrease in labeling of the PCA motoneurons in their freeze-regeneration study. They also reported chromatolytic changes in PCA neurons, but suggested the decrease in labeling was due not to neuronal death but to the inability of the new axons to transport HRP. Studies to determine the ability of the regenerated RLN to retrogradely transport dye, as well as investigate the question of cell death and selective reinnervation, are in progress.

SUMMARY

Fluorescent dyes were used for retrograde neuronal labeling studies in normal rats and rats that

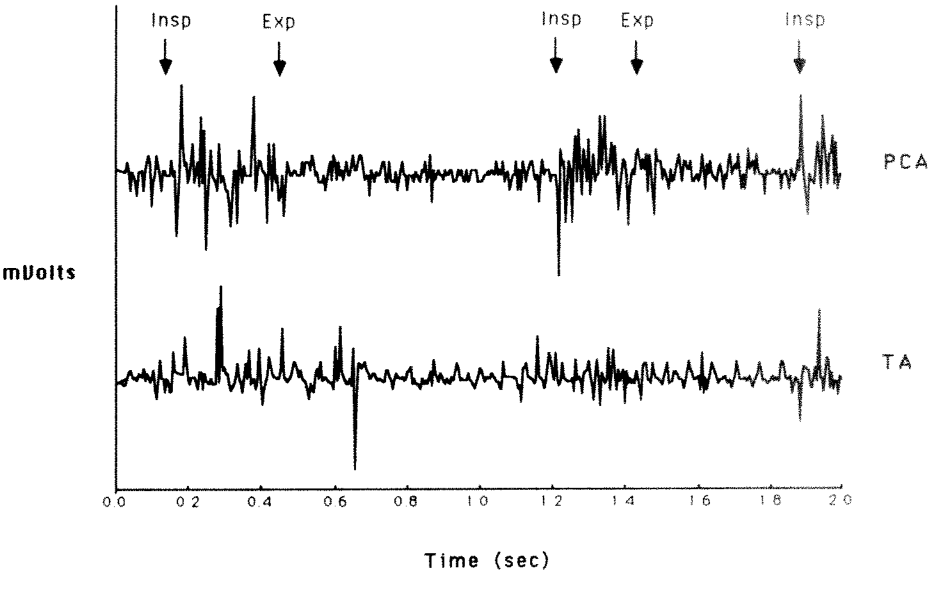

Fig 11. Simultaneous tracings of PCA and TA laryngeal electromyographic recordings are shown 3 months following reinnervation. Phasic electrical activity synchronous with respiration is seen in both TA and PCA tracings. Insp — inspiration, Exp — expiration.

underwent a reinnervation procedure of the RLN. Normal animals were found to have a functional organization of the laryngeal motoneurons in the NA. Reinnervated animals lost this organization in the NA, and the diminished population of reinnervating neurons were found in a location similar to that of the adductor neurons in the NA of normal control animals.

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COCHLEAR IMPLANTS: HISTOPATHOLOGIC FINDINGS RELATED TO PERFORMANCE IN 16 HUMAN TEMPORAL BONES

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This paper presents results of a histologic study of 16 temporal bones with cochlear implants from 13 subjects. Damage caused by electrode insertion in the basal turn of the cochlea was evaluated. Dendrite and spiral ganglion cell populations were compared to clinical performance scores to determine structures necessary for stimulation and the minimum number needed for electrical stimulation. Results show that damage from insertion of long electrodes was located mainly at the most anterior part of the basal turn; that despite total degeneration of dendrites in the area near the electrode, some spiral ganglion cells remained; and that spiral ganglion cells or possibly axons are the stimulated structures and that fewer of them than previously thought are necessary to achieve a hearing sensation from electrical stimulation.

KEY WORDS — cochlear implant, spiral ganglion cell count.

INTRODUCTION

This paper presents histologic findings in 16 temporal bones with cochlear implants from 13 subjects. We evaluated basal turn damage caused by the insertion of the electrode; estimated dendritic populations in the basal, middle, and apical turns of the cochlea; and counted spiral ganglion cells in all bones. We compared estimates of the amount of dendrites and actual counts of spiral ganglion cells to clinical performance scores to determine structures necessary for electrical stimulation and the minimum number needed for stimulation. Other authors have reported spiral ganglion cell counts in cochleas from patients with normal audiograms or with profound sensorineural hearing loss.[1-3]

MATERIALS

We considered 24 temporal bones from 13 subjects for this study. Two bones were excluded because the cochlea on the nonimplanted side was transected when removed. Of the 13 subjects, 9 had bilateral bones: 3 with bilateral implants and 6 with unilateral implants. In the remaining 4 cases, only the implanted bone was available. Thus, we studied 16 implanted ears and 6 unimplanted ears. Table 1 lists the type and length of insertion of electrode.

TABLE 1. CLINICAL DATA ON 16 IMPLANTED TEMPORAL BONES

Case	Sex	Age	Cause of Deafness	Implant		Duration (y)	
				Type	Length (mm)	Disease	Electrical Stimulation
1	M	55	Intralabyrinthine neuroma	3M/House	6	7	3.0
2	M	67	Otosclerosis	3M/House	6	55	2.5
3	M	71	Undetermined	3M/House	6	62	4.5
4	M	75	Otosclerosis	3M/House	20	60	14.0
5	M	44	Undetermined	3M/House	18	8	5.0
6	M	47	Otosclerosis	Ineraid	22	15	5.0
7	F	60	Undetermined	3M/House	18	30	8.5
8	M	69	Ototoxic drug	3M/House	18	5	2.0
9	M	54	Fracture	3M/House	18	31	2.0
10	M	60	Otosclerosis	3M/House	16	20	1.0
11R	F	61	Undetermined	3M/House	16	18	6.0
11L				Nucleus	22		1.0
12R	F	59	Undetermined	3M/House	16	46	3.5
12L				3M/House	16		9.0
13R	F	58	Ototoxic drug	3M/House	16	28	10.0
13L				3M/House	6		1.0

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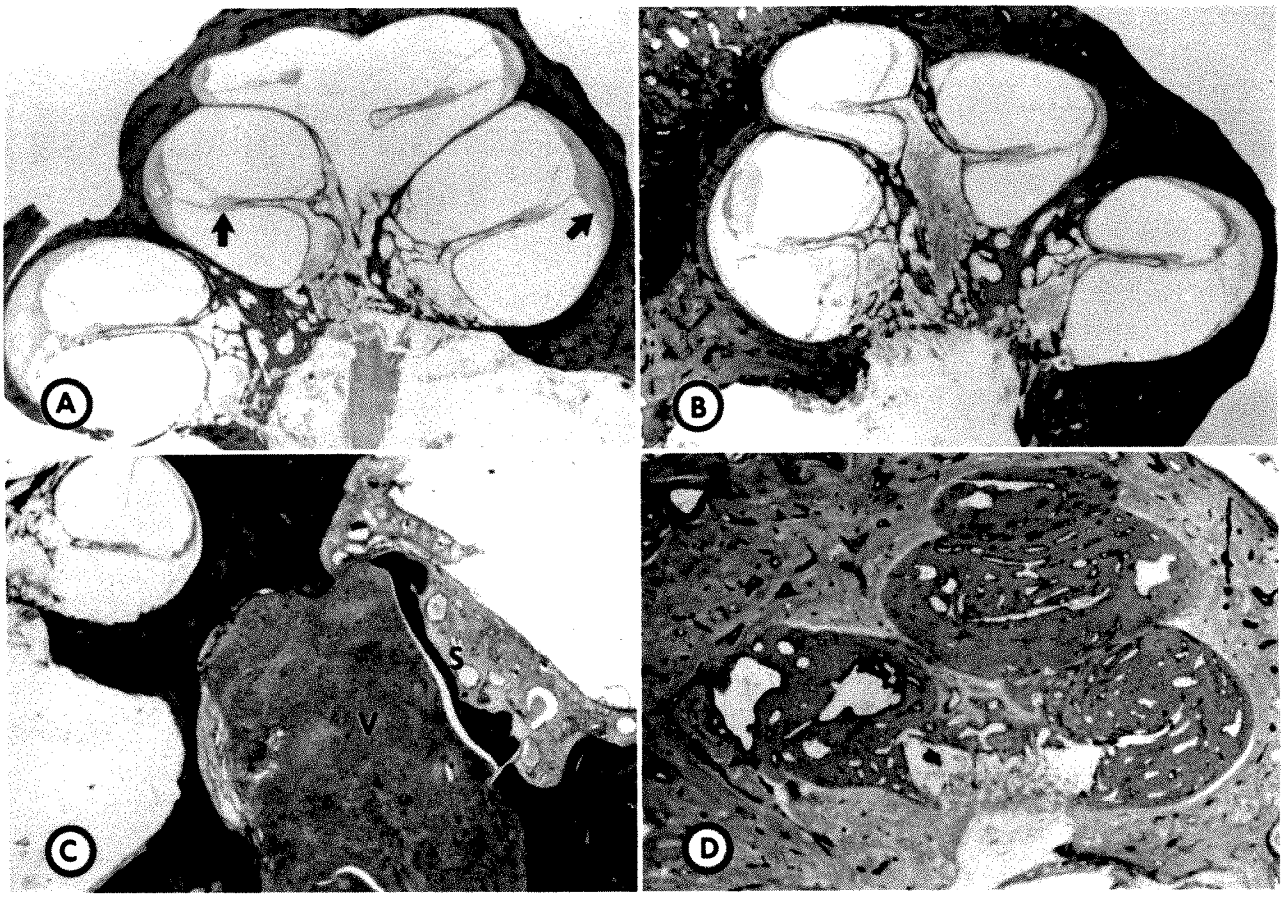

Fig 1. Histologic findings. A) (Case 3) Amorphous degeneration (arrows) of organ of Corti, of stria vascularis, and of all vestibular sensory elements. B) (Case 2) Cochlear otosclerosis. C) (Case 1) Intralabyrinthine neuroma filling vestibule (V) and displacing stapes (S). D) Labyrinthitis ossificans.

There were nine men and four women. Ages ranged from 44 to 75 years, with a mean of 60 years. Duration of disease ranged from 5 to 62 years. Duration of electrical stimulation ranged from 1 year to 14 years. The cause of deafness was otosclerosis in four cases, ototoxicity in two cases, temporal bone fracture in one case, and undetermined in five cases; and an intralabyrinthine neuroma was found on the implanted side at the autopsy of one case (Fig 1C and Table 1).

METHODS

All temporal bones were fixed in formalin, decalcified in ethylenediaminetetraacetic acid, and embedded in celloidin. Nineteen bones were sectioned at a thickness of 20 μm in the horizontal plane; two bones (sectioned elsewhere) at 6 μm in the horizontal plane (cases 8 and 10 on the implanted side); and one bone (case 4 on the implanted side) was sectioned in a vertical plane (sections are parallel to the plane defined by the basal turn of the cochlea) because of technical difficulty in removing the electrode from the bone. Every 10th section was stained with hematoxylin and eosin and mounted on glass slides for all bones except two, with few spiral ganglion cells, of which all sections were stained and mounted. Damage caused by electrode insertion was evaluated by light microscopy.

To compare dendrite populations with remaining spiral ganglion cells, one of us (F.H.L.) with 40

TABLE 2. PERCENT CORRECT TEST SCORES

	Monosyllable, Trochee, Spondee		
Case	*Word*	*Stress*	*Environmental Sounds*
1	33	92	60
2	29	71	80
3	58	96	75
4	42	100	50
5	33	75	45
6*			
7	50	92	40
8	25	63	70
9	NA	NA	63
10	NA	NA	57
11R	42	83	95
L	83	100	55
12R	42	75	70
L	46	88	75
13R	66	100	70
L	54	83	80

NA — not available.

*Speech recognition scores of this patient, with Ineraid device, on open-set, and unpracticed word lists of 36 two-syllable words read in random order, were 64% with implant alone and 94% with lipreading.

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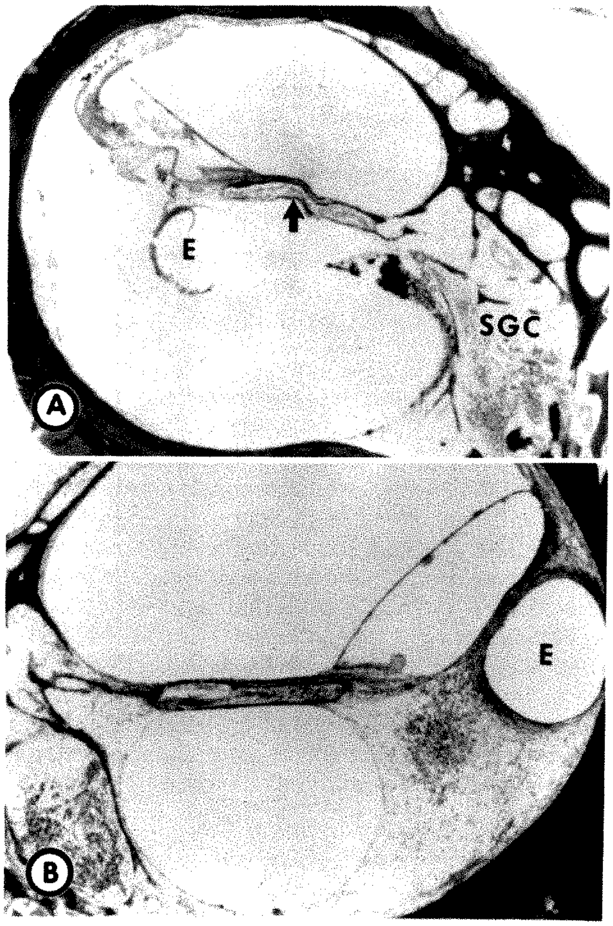

Fig 2. (Case 11L) Disruption caused by electrode (E) insertion. A) Anterior part of basal turn. Note fracture of osseous spiral lamina (arrow), dendrite degeneration and corresponding remaining spiral ganglion cell population (SGC), disruption of stria vascularis, displacement of basilar membrane, and absence of organ of Corti. B) Tip of E dissecting stria vascularis from cochlear wall.

years' experience in temporal bone histopathology estimated the population of dendrites in the apical, middle, and basal turns of the cochlea. The result is averaged and reported as a percentage of normal. With the aid of an ocular grid, we also counted the spiral ganglion cell populations in all 22 bones according to the method described by Guild[4] and Otte et al.[5] For each temporal bone, we multiplied each count by a factor of 10 to account for unmounted sections, and then by Konigsmark's correction factor to account for doubly counted spiral ganglion cells. This correction factor is 0.91 for a 20-μm-thick section and 0.75 for a 6-μm-thick section.[6] In two cases (9 and 12R) with few spiral ganglion cells, we stained all sections with spiral ganglion cells and counted them. Thus, we are reporting total ganglion cell counts for each bone. We did not graphically reconstruct the organ of Corti, since it was destroyed by the long electrodes in the anterior part of the cochlea.

To determine which anatomic structures are necessary for stimulation, we compared the estimate of dendrites and the number of spiral ganglion cells to clinical performance scores on the Monosyllable, Trochee, Spondee (MTS) Word and Stress Tests and the Environmental Sounds Test (Table 2). They were the only available tests for the majority of patients, who were early implant users (1970s) from the Otologic Medical Group or from coinvestigators.

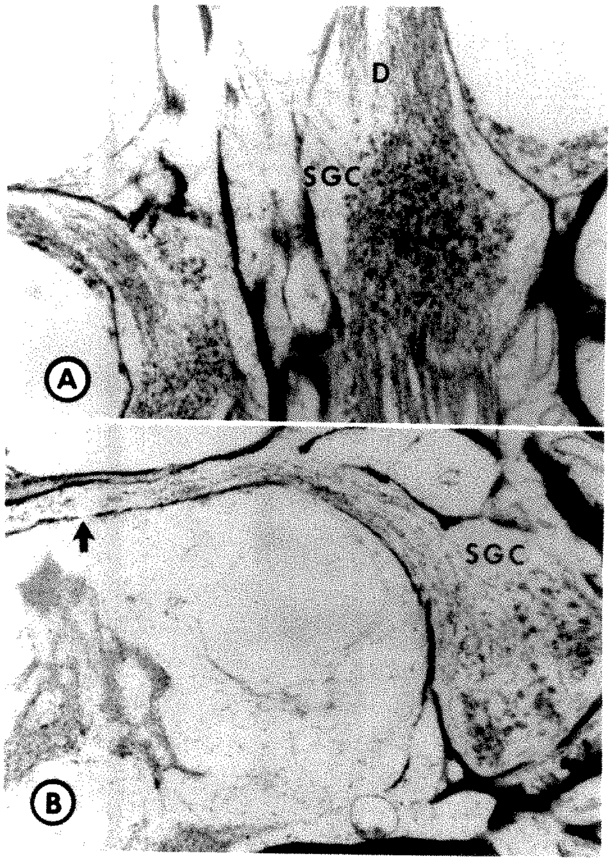

Fig 3. (Case 11L) Neuronal populations. A) Populations of dendrites (D) and spiral ganglion cells (SGC) are close to normal in upper part of spiral ganglion, distal to tip of electrode. B) Despite degeneration of dendrites (arrow) in front of electrode in basal turn of cochlea, some SGCs still remain in Rosenthal's canal.

For information on the minimal number of spiral ganglion cells needed for electrical stimulation, the clinical performance of subjects with few spiral ganglion cells was compared to the clinical performance of 86 postlingually deaf single-channel users on the same tests.

RESULTS

Damage in Basal Turn of Cochlea. With long electrodes, damage occurred at the anterior part of the basal turn where the electrode hits the outer wall of the cochlea and then bends toward the modiolus. At this level we saw disruption of the spiral ligament and stria vascularis, resorption of the organ of Corti, ossification from the damaged endosteum,[7,8] fracture of the osseous spiral lamina, and degeneration of dendrites (Fig 2). There was no corresponding total loss of spiral ganglion cells (Fig 3).

TABLE 3. DENDRITES AND SPIRAL GANGLION CELL POPULATIONS

Case	*Side*	*Percentage* of Dendrites*	*No. of Spiral Ganglion Cells*
1	Implanted	45	15,255
2	Implanted	30	10,365
	Unimplanted	5	7,025
3	Implanted	0	16,352
	Unimplanted	0	16,835
4	Implanted	0	6,194
	Unimplanted	0	4,504
5	Implanted	35	15,898
	Unimplanted	48	18,473
6	Implanted	0	9,218
	Unimplanted	77	10,702
7	Implanted	18	8,873
	Unimplanted	48	NA
8	Implanted	47	16,620
9	Implanted	0	3,212
	Unimplanted	0	8,463
10	Implanted	15	14,205
	Unimplanted	8	NA
11	Implanted	77	21,430
	Implanted	58	18,555
12	Implanted	0	3,376
	Implanted	40	18,646
13	Implanted	0	12,804
	Implanted	0	9,364

NA — not available (cochlea was transected).
*Average of percentages for each cochlear turn.

Comparison of Dendrite and Ganglion Cell Populations. Table 3 shows estimates of dendrite populations in all turns of the cochlea and the corresponding total spiral ganglion cell counts. The population of dendrites in implanted and nonimplanted ears ranged from none to 77%, with comparable averages (23% unimplanted and 23% implanted).

The spiral ganglion cell population ranged from 3,212 to 21,430. The mean in unilaterally implanted cases on the implanted side was 11,619 and on the nonimplanted side, 11,000. In bilaterally implanted patients, the mean was 14,029.

Total dendrite absence is compatible with a good ganglion cell count. One case (case 3) with total bilateral disappearance of dendrites had almost the same number of spiral ganglion cells on both sides (16,835 unimplanted and 16,352 implanted). This case demonstrated that ganglion cells can occur in areas of total dendrite degeneration. The mean number of spiral ganglion cells in implanted and unimplanted bones with remaining dendrites is higher than in bones with a completely absent dendrite population (Table 4).

TABLE 4. COMPARISON OF PRESENCE OR ABSENCE OF DENDRITES AND MEAN NUMBER OF SPIRAL GANGLION CELLS IN 22 TEMPORAL BONES

	Spiral Ganglion Cells	
Dendrites	*Nonimplanted Ears*	*Implanted Ears*
Absent	9,934 (n = 3)	8,686 (n = 7)
Present	12,066 (n = 3)	15,757 (n = 9)

TABLE 5. COMPARISON OF PRESENCE OR ABSENCE OF DENDRITES AND CLINICAL PERFORMANCE

	Monosyllable, Trochee, Spondee		
Dendrites	*Word*	*Stress*	*Environmental Sounds*
None (n = 6 ears)	52	91	68
Present (n = 8 ears)	37	80	65

Ears had single-channel implants. Data are percent correct.

Structures Necessary for Stimulation. Seven of 16 implanted ears had no dendrites; 7 had less than 50% of the normal dendrite population; and 2 had more than 50%. All implanted ears, with or without dendrites, were from successful cochlear implant users. Table 5 shows the performance of single-channel users in ears with and without dendrites. We did not find an association between the presence or absence of dendrites and the clinical performance of these patients on these tests. These findings suggest that dendrites do not aid the auditory perception of single-channel cochlear implant patients. It appears that spiral ganglion cells or possibly axons are the stimulated structures.[9,10]

Minimum Number of Spiral Ganglion Cells Necessary for Stimulation. There was no association between the number of spiral ganglion cells and clinical performance. Factors other than the spiral ganglion cell count may be relevant. Two implanted ears had only 10% of the normal number of spiral ganglion cells, considered to be 30,000. These two subjects' clinical performance on the Monosyllable, Trochee, Spondee Word and Stress Tests and the Environmental Sounds Test was as good as the average performance of 86 postlingually deafened adults (Table 6). These two ears might illustrate that fewer ganglion cells than previously thought are necessary for stimulation.

CONCLUSIONS

Our findings suggest that the structures stimulated by the cochlear implant are essentially the spiral ganglion cells or the axons; that the insertion of the electrode does produce dendrite degeneration in

TABLE 6. COMPARISON OF SCORES OF 86 ADULTS WITH SINGLE-CHANNEL IMPLANTS AND OF TWO STUDIED CASES WITH LOW SPIRAL GANGLION CELL COUNT

	Monosyllable, Trochee, Spondee		
	Word	*Stress*	*Environmental Sounds*
Average (n = 86)	33	75	55
Chance	8	33	20
Case 12R	42	75	70
Case 9	NA	NA	63

Data are percent correct.
NA — not available.

areas near the electrode but no corresponding total reduction of the spiral ganglion cell population; and that response to stimulation may occur with as little as 10% of the normal population of ganglion cells.

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INTRACOCHLEAR POTENTIAL DISTRIBUTION WITH INTRACOCHLEAR AND EXTRACOCHLEAR ELECTRICAL STIMULATION IN HUMANS

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Intracochlear potential distributions were measured from subjects implanted with the Ineraid multichannel cochlear implant. The electrode array provided direct accessibility for obtaining measurements. Different modes of stimulation were used. The findings with intracochlear monopolar stimulation seem to confirm that significant low-resistance pathways, in which currents can flow into or out of the cochlea, are located in the basal turn. The results with extracochlear stimulation only show small intracochlear potential gradients. This finding suggests that no substantial currents flow along the auditory nerve fibers when extracochlear stimulation is used. These results concur with the electrically evoked auditory brain stem responses, in that intracochlear stimulation consistently elicited auditory brain stem responses, whereas responses elicited at or near the round window by extracochlear stimulation were weak or absent.

KEY WORDS — cochlear implant, electrical stimulation, evoked auditory brain stem response, intracochlear current flow.

INTRODUCTION

Multichannel cochlear implants are generally designed to elicit auditory nerve activity at different locations in the cochlea. Regardless of the speech-processing strategy, full benefit of a multichannel device may depend on the distribution and number of surviving auditory nerve fibers. Electrophysiologic techniques could be valuable to estimate neural survival in cochlear implant candidates. Animal studies show a variety of results when relating electrically evoked auditory brain stem response (EABR) data to spiral ganglion cell survival.[1-6] Since it has been reported that the position of the stimulating electrode affects electrophysiologic recordings,[7] it is worthwhile to study how the electrode positioning and the resulting current spread affects the efficiency of electrical nerve stimulation and subsequently the amplitudes of the brain stem responses.

This work reports measurements of the intracochlear potential distribution and of electrophysiologic recordings demonstrating the activity of the peripheral auditory system. Electrical stimulation was applied with electrodes placed inside and outside the cochlea. We first examine recordings of EABR using different locations for the electrodes; second, we examine the intracochlear potential distributions for these electrode sites; and finally, we discuss the implications of a design for preoperative testing that could assess the status of the peripheral auditory system in potential cochlear implant candidates.

METHODS

Subjects. Data from 10 subjects that can be divided into two groups, consisting of 5 patients each, are presented. Group A consists of 3 subjects implanted with the Ineraid electrode array and 2 subjects tested with a single flame-ball electrode inserted through the ear canal into the scala tympani. Everyone in this group was tested under general anesthesia during surgery for implantation. All 5 subjects had round window stimulation prior to operation on the inner ear. Group B consists of 5 subjects already implanted with the Ineraid electrode array. The subjects were not anesthetized. None of them showed apparent ossification of the inner ear at the time of operation, and the electrode array was fully inserted.

Electrophysiologic Recordings. The EABRs were recorded with silver-silver chloride electrodes between the forehead (positive electrode) and the mastoid ipsilateral to the stimulated ear (negative electrode). During retroauricular operation the negative electrode was placed on the ipsilateral cheekbone. An electrocardiogram self-adhesive electrode was placed on the shoulder and served as a ground. The subjects (group A) were under general anesthesia. The equipment for stimulus generation and the stimulus waveform were exactly the same as described by Pelizzone et al.[8] Stimulus duration for four of the five subjects was set to 100 microseconds per phase (for subject JS, 500 microseconds per phase). The repetition rate was 11.1/s. Stimulus intensity was expressed in microamperes peak-to-peak (μAp-p). Extracochlear stimuli were applied between a platinum flame-ball electrode placed on the membrane of the exposed round window and the far-field ground on the shoulder. Intracochlear stimuli were applied either between the most apical electrode (No. 1) of the Ineraid electrode array and the most

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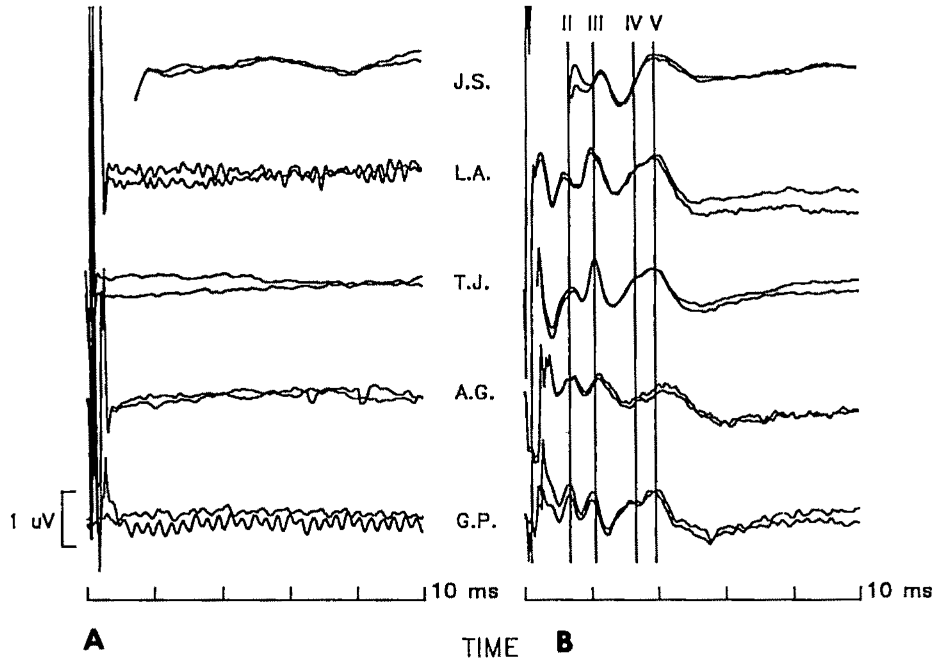

Fig 1. Comparison of electrophysiologic recordings with different stimulation sites from five subjects (group A) under general anesthesia. A) Initial 10-millisecond recordings after stimulus presentation at round window. Recordings were obtained at 1 mAp-p current magnitude. B) Electrically evoked brain stem responses recorded from same subjects during same operation with scala tympani electrode stimulation. Current magnitudes ranged between 500 μAp-p and 900 μAp-p.

basal electrode (No. 6, usually about 2 mm beyond the round window) or between an intracochlear single platinum flame-ball electrode inserted at least 20 mm into the scala tympani and the far-field ground electrode on the shoulder. Single ball electrodes (diameter 0.5 mm) were made from Teflon-insulated platinum wire (0.125 mm diameter). The recordings were obtained using equipment that has been previously described.[8,9] By analogy with conventions established for acoustically evoked brain stem potentials in humans, vertex-positive peaks were labeled with roman numerals.[10]

Intracochlear Potential Measurements During Electrical Stimulation. Intracochlear potential distributions were measured in subjects of group B, implanted with the Ineraid intracochlear electrode array, consisting of eight platinum ball electrodes (0.5 mm diameter), six of which are placed in the scala tympani with 4-mm spacing. One was located on the promontory (No. 7) and another placed under the temporal muscle (No. 8). During the testing session an additional electrocardiogram self-adhesive electrode was attached to the ipsilateral arm. All postoperative tests took place at least 6 weeks after implantation. A constant current stimulus was presented at a repetition rate of 20/s to one pair of electrodes. The stimulus waveform was exactly the same as used for EABR measurements, except that initial phase polarity was not altered. The stimulus intensity was set to 50 μA root mean square (rms) and was inaudible for all tested subjects. Potential waveforms were recorded between all combinations of the nonstimulated electrode pairs. The recordings were amplified (gain 100), digitized (20 kHz), averaged (25 sweeps), and saved on disk for later processing. The rms magnitude for each recorded waveform was calculated off-line and processed into a 9 x 9 data matrix. The columns of the matrix represent the "positive" recording electrodes and the rows represent the "negative" recording electrodes. All data entries are expressed in millivolts rms potential difference calibrated to a 20-μArms stimulation current. Diagonal entries were set to zero (values were below noise floor of the measurement equipment — typically less than 0.15 mVrms). Entries below the diagonal were multiplied by -1 after checking that corresponding waveforms had inverted polarity compared to waveforms corresponding to the above diagonal values. Each row of the data matrix corresponds to a potential distribution curve referenced to the specified electrode. Calculating the mean of this series of curves renders the resulting potential distribution independent of any reference electrode. All potential distribution plots presented in this study are reference-independent curves.

The measurement protocol was fully automatized with the help of a personal computer communicating over an IEEE-488 connection (Hewlett Packard IB) with a control unit (HP 3852A) equipped with a relay multiplexer module (HP 44705A) to switch the different electrode pairs automatically. All electrical connection lines between the subject and the testing equipment were optically decoupled for safety reasons. A test run lasted 12 minutes and was repeated twice for each stimulation configuration.

RESULTS

Intraoperative Electrophysiologic Recordings. Figure 1A shows recordings of the first 10 milliseconds from 5 subjects (group A) after presentation of the electrical stimulus delivered by a single platinum flame-ball electrode to the round window. Stimulus intensity was 1 mAp-p for all record-

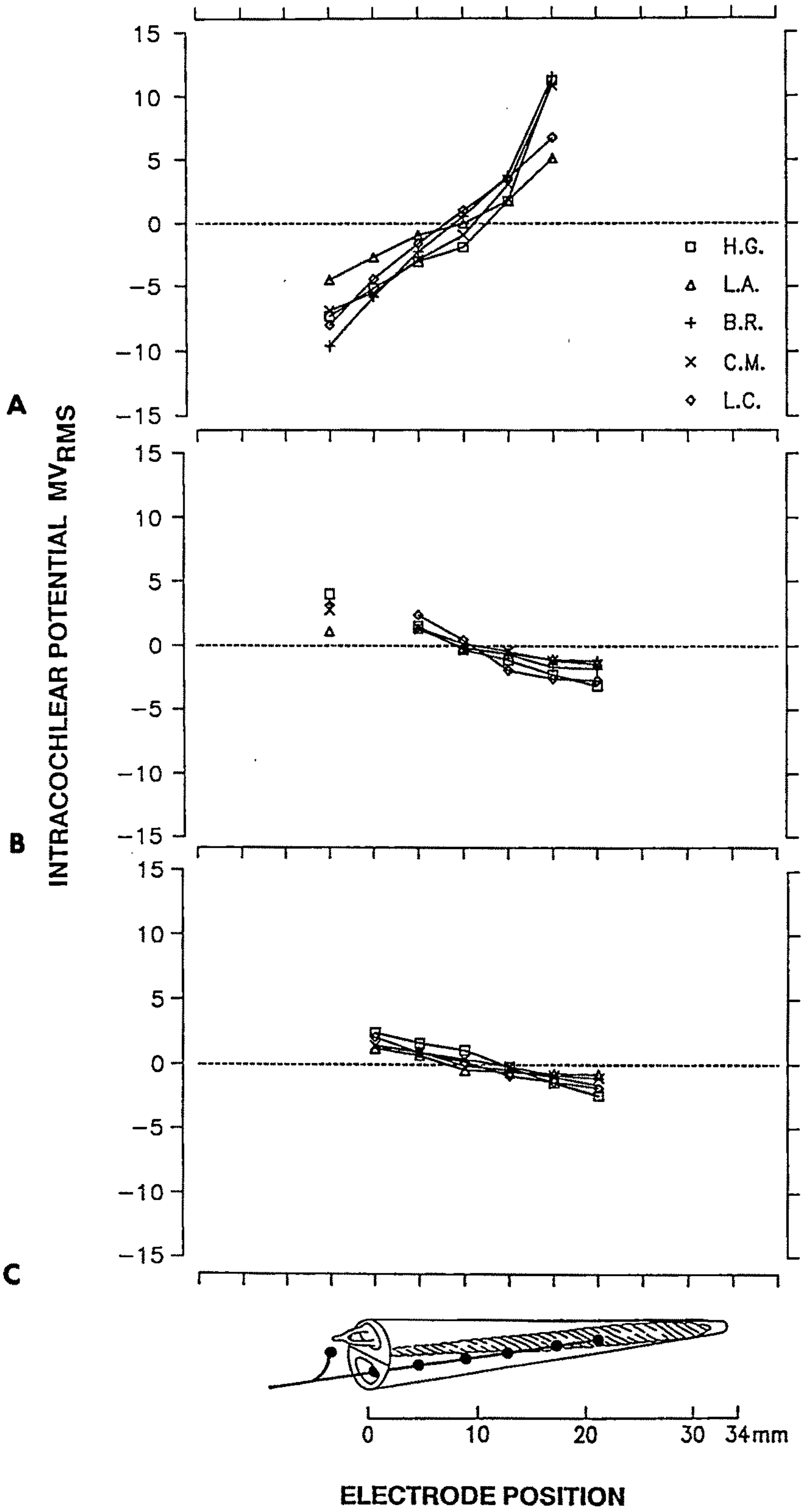

Fig 2. Intracochlear potential distribution measurements from five subjects (group B) implanted with Ineraid intracochlear electrode array. A) Apical electrode stimulation, 20 to 24 mm inside scala tympani (No. 1). B) Basal electrode stimulation, 0 to 4 mm inside scala tympani (No. 6). C) Extracochlear electrode stimulation on promontory (No. 7). Potentials are expressed in millivolts rms and were calibrated to 20 μArms stimulus current.

ings with extracochlear stimulation. No apparent evoked responses could be detected with this current strength. Any obvious technical problem could be excluded. Figure 1B shows EABRs recorded from group A subjects after opening the cochlea and applying intracochlear stimulation. The scala tympani electrode was 20 to 24 mm inside the cochlea as measured from the round window. Recordings from subjects JS and GP were elicited by stimulation with a single platinum flame-ball electrode, while the responses on subjects LA, TJ, and AG were evoked by stimulating between the most apical electrode (No. 1) and the most basal electrode (No. 6) of the Ineraid electrode array. Stimulus intensity was set to 500 for JS, 750 for JA, TJ, and AG, and 900 μAp-p for GP. The characteristic series of vertex-positive peaks of the EABR was consistently elicited in spite of the different current magnitudes and different intracochlear electrode configurations. Mean latencies of waves II, III, IV, and V were 1.34, 2.1, 3.3, and 3.91 milliseconds, respectively. The shapes of the waveforms compare nicely to those of already reported studies on humans, and latencies are in good agreement with data reported by others.[11-14]

Intracochlear Potential Distribution Measurements. Intracochlear potential distribution measurements in the scala tympani from the five subjects in group B are shown in Fig 2. Figure 2A,B,C presents the potential distributions measured for apical (No. 1), basal (No. 6), and promontorial stimulation sites, respectively. Potential measurements were made on one subject for different current strengths and showed a linear relationship between potential magnitudes and stimulating current up to 200 μArms. Measurements with steady state sinusoidal stimuli at different frequencies give support to the notion that intracochlear currents flow primarily through resistive pathways.[15] We therefore calibrated all the potential measurements by a linear factor to 20 μArms stimulus input. Evident differences in potential gradients are observed for stimuli applied at the most apical and the most basal electrodes. Stimulation on the promontory results in very small gradients. Gradients differ by a factor of 3 between apical and basal stimulation and by a factor of 4 between apical and promontorial stimulation.

DISCUSSION

Measurements of intracochlear potential distributions showed a marked difference between apical and basal stimulation. Since the slope of the potential distribution curve is directly proportional to the magnitude of current flow, the steep gradients observed for apical stimulation suggest a large current flow toward the base. The small gradients observed for basal stimulation suggest that little current flows toward the apex of the cochlea. The even smaller gradients obtained with extracochlear stimulation suggest that only marginal currents flow in the cochlea. The measurements seem to confirm the proposed qualitative electroanatomic model[16] that suggests significant current flow in or out of the cochlea through basal located openings. Although this model was proposed to explain the pathways of single unit action currents that sum up to gross neural potentials observed at the round window, it may also serve as an explanation of current pathways for applied electrical currents that activate these neurons. Significant pathways of current flow between the stimulating intracochlear electrode and an extracochlear return electrode coincide with the presumed pathways of single unit action currents of many nerve fibers located basalward from the active electrode.

The observation of clear EABR with an intracochlear stimulation that produces large intracochlear current flow suggests that many auditory nerve fibers are synchronously stimulated. The small intracochlear potential gradient produced by basal electrode stimulation demonstrates that current flow between the basal region of the cochlea and a far-field ground in the temporal muscle does not have its main pathway through the inner ear. In addition, we were unable to record EABR with round window stimulation. Previously reported findings from the cat[7] are consistent with our observations. Although spike activity of auditory nerve fibers would best be reflected in the compound action potentials,[17,18] represented by wave I of the brain stem responses,[19] absence of the later waves is a clear indication that no input to the brain stem structures was present.[20] We therefore conclude that round window stimulation produces little or no activity in the auditory nerve fibers.

The slope of the intracochlear potential distribution was found to be directly proportional to the stimulus current applied in the cochlea. This is not necessarily true for stimulation between extracochlearly located electrodes, since the current pathways are obviously not the same. However, it seems conceivable that one can induce steeper potential gradients with a round window electrode by using larger currents. A rough estimation based on the present measurements suggests that a current at least five times higher is needed to obtain a gradient comparable to intracochlear stimulation. Although such high currents would still be below current densities that produce gassing at the electrode surface,[21] they would certainly be painful for the subjects. The clinical utility of such stimulation is therefore limited.

The survival of auditory nerve endings and their optimal distribution in the cochlea appears to be of increasing importance as multichannel cochlear implants become more widely used. Recordings of the first wave of the brain stem response directly reflect the activation of single nerve fibers and would therefore be the predictor of choice to evaluate preoperatively spiral ganglion cell survival in cochlear implant candidates. Amplitude measurements on the first wave and slopes of growth functions seem to correlate best with spiral ganglion cell survival in animal studies.[6] Since the electrical artifact obscures recordings of wave I of the EABR in humans, amplitude measurements of later brain stem wave could still give some indication of the status of surviving nerve endings. However, it seems important, when evaluating the status of the peripheral nerve endings with electrophysiologic recordings, to apply stimuli in a manner in which most of the surviving nerve endings are stimulated. This seems to be best accomplished with a monopolar electrode placed as deep as possible in the cochlea.

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Ann Otol Rhinol Laryngol 100:1991

PSYCHOLOGICAL PREDICTORS OF AUDIOLOGICAL OUTCOMES OF MULTICHANNEL COCHLEAR IMPLANTS: PRELIMINARY FINDINGS

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The purpose of this research was to determine whether psychological variables were associated with the variability that characterizes the audiological performance of recipients of multichannel cochlear implants. Twenty-nine consecutive recipients of multichannel implants participated in a preoperative psychological assessment and audiological follow-up assessments after 18 months of implant use. Experimental cognitive measures that assess an ability to rapidly detect and respond to features imbedded in sequentially arrayed information accounted for up to 30% of the variance in implant outcome, suggesting the importance of cognitive abilities in implant outcome. Standardized measures of intellectual ability, however, were not predictive of outcome. The Health Opinion Survey, a measure of participatory engagement, was also a significant predictor of audiological outcome. Overall, the results implicated the importance of several specific psychological factors in the audiological outcome of cochlear implants in postlingually deafened adult recipients.

KEY WORDS — audiology, multichannel cochlear implant, psychological predictors.

There is convincing evidence that the current generation of cochlear implants can result in significant audiological gain and improved ability to receive aural communication.[1-4] It is also clear, however, that the performance of patients with cochlear implants is extremely variable. Indeed, the recent National Institutes of Health Consensus Development Conference statement on cochlear implants suggests that few medical interventions are associated with as variable an outcome as cochlear implants.[5] Although there is evidence that multichannel devices are superior to single-channel devices,[3] even within multichannel systems variability continues to be a ubiquitous characteristic of implant outcome. As a result, understanding the variability and predicting the performance of individual cochlear implant recipients are research and clinical challenges.

In efforts to understand the variability in audiological outcome, psychological variables have been considered likely candidates.[6-8] The possibility that psychological factors could be involved in implant outcome is predicated, in part, on the recipients' apparent need to learn to use the implant and the lengthy period that is required to gain maximum benefit, as well as clinical impressions that motivation and active engagement are needed to achieve maximum benefit. The apparent need to learn to use the implant signal suggested the possible utility of intellectual and cognitive measures, while the need for active participation and engagement suggested the possible utility of personality attributes as predictors of implant outcome.

The apparent utility of standardized measures of general intelligence has resulted in their inclusion in establishing patient suitability for a cochlear implant in some programs, yet no evidence has been provided to document the utility of measures of general intelligence as predictive of cochlear implant outcome. Similarly, no standardized general measures of personality have been established as useful in understanding variability in implant outcome. While that state of affairs could be used to argue that psychological variables may not be useful as predictors of cochlear implant outcome, it may be that the existing evidence merely reflects the selection of the wrong measures. Indeed, it has been suggested that the usual standardized psychological measures are inadequate for the task of evaluating profoundly hearing-impaired implant candidates with respect to variables relevant to implant success.[9]

The present research was designed to determine whether several specific, rather than general, psychological attributes of cochlear implant recipients might be predictive of the outcome of multichannel implants. Because of the apparent learning necessary to achieve benefit from the implant, it seemed likely that some specific cognitive abilities might be involved in implant use, rather than the more general intellectual abilities usually measured. That is, it was hypothesized that attentional processes, the ability to identify changes in rapidly occurring sequentially arranged events, and rapid information-processing abilities might be important in implant use. For that reason, a series of experimental cognitive tasks were developed. Each of these tasks was selected to assess the implant candidate's ability to

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REPRINTS — John F. Knutson, PhD, Dept of Psychology, The University of Iowa, Iowa City, IA 52242.

identify features from sequentially arrayed information and to respond rapidly upon the identification of those features. In addition to these experimental measures, the Raven's Progressive Matrices[10] were included. Although the Raven's was developed as a nonverbal measure of general intelligence, it was selected for the present study to assess the ability of subjects to identify sequential patterns. The Raven's presents subjects with a 3 x 3 matrix of geometric patterns that change systematically in two dimensions. One cell is missing and the subject must identify the rules governing changes in the two-dimensional pattern of the matrix and select the missing component from among eight alternatives. Recently, Carpenter et al[11] directly assessed the processes underlying the Raven's task by using eye tracking measures and oral records of subjects while they completed testing. These process data provided by Carpenter et al[11] suggest that the Raven's might be useful in predicting implant outcome if a measure of pattern recognition and sequence learning is relevant to the use of an implant.

Because a successful response to some medical interventions requires considerable compliance and participation by the patient, and because compliance with medical procedures may be a context-specific attribute,[12,13] it was hypothesized that success with an implant may be a function of medical compliance. Consequently, the Health Opinion Survey[14] was included in the present research as an index of the subject's opinion of and desire for participation in his or her own health care.

METHOD

Subjects. Subjects were 29 consecutively implanted multichannel cochlear implant recipients who, at the time of this analysis, had completed 18 months of implant use as participants in the Iowa Cochlear Implant Program. To be eligible for a cochlear implant in the research program, subjects were required to evidence a profound (pure tone average >95 dB hearing level) bilateral, postlingually acquired hearing loss and receive marginal benefit from hearing aids. Subjects ranged in age from 22 to 70 years, with a mean of 52.1 and an SD of 14.12. None scored higher than 4% correct when NU-6 word lists were presented at 60 dB hearing level in the sound field with an appropriately fitted hearing aid. Under aided conditions, none understood more than 10% of the words from the auditory-only presentation of the Sentences Without Context Task (Sentences) from the Iowa Phoneme and Sentence Test.[15]

Because the Iowa cochlear implant research protocol entails regularly scheduled follow-up sessions for 5 years, and because many of the test procedures require research participants to be literate and to be able to respond to a broad range of test materials, in addition to medical and audiological tests, several psychological variables were included among criteria used to select research participants. Namely, implant candidates could not present with a severe psychiatric illness (eg, schizophrenia) or a personality disorder that would reduce the likelihood of participating in the follow-up protocol.

Because of the demands of the research protocol, subjects were also required to evidence measured intelligence within or above the low-average range. Based on the Wechsler Adult Intelligence Scale–Revised (WAIS-R; administered without the Arithmetic and Digit Span subtests), the Full Scale IQ Score of the sample ranged from 82 to 132, with a mean of 107.6, and an SD of 13.7. It is important to note that these psychological selection criteria were predicated on the demands of the research protocol and not on their possible relevance to implant outcome. Although the criteria for admission to the research protocol truncate the range of IQ scores at the lower end of the distribution, the obtained sample evidenced a broad range of intellectual abilities and only subjects more than 1.6 SD below the general population mean were not represented. Since 1982, 99 postlingually deafened adults seeking a cochlear implant at Iowa have participated in preoperative psychological evaluations. The WAIS-R Full Scale IQ of that total sample ranged from 72 to 138 with a mean of 103.3 and an SD of 14.7, so the intellectual characteristics of the present sample are not unlike those of the population of postlingually deafened adults seeking an implant at The University of Iowa Hospitals and Clinics.

Procedure. Candidates presenting for a cochlear implant participate in extensive audiological, medical, radiographic, and psychological assessments distributed over 4 days. This preimplant evaluation is completed no more than 3 months prior to the scheduling of the operation for subjects who receive a cochlear implant in the research program. Subjects who were to receive a cochlear implant in the program were assigned to receive either the 21-channel pulsed signal Nucleus implant[16] or the 4-channel Ineraid analog signal device,[17] following an alternating assignment.

The preimplant psychological evaluation in the current project was typically scheduled after subjects had met the audiological, medical, and radiographic criteria for implant eligibility at Iowa. Thus, the psychological evaluation was the final stage of the preimplant assessment procedure; it entailed a full day of tests, interviews, and questionnaires. The assessments included variables suitable for screening subjects for the research protocol and variables suitable for monitoring change in psychological functioning following the use of the implant, as well as the psychological predictor variables that are the focus of the present research.

Psychological Predictor Measures. The experimental cognitive tasks were selected to be visually presented analogs of the tasks that seem to confront the implant recipient. Namely, the implant recipient must be able to rapidly identify stimuli that are imbedded in a sequence of continually changing signals. Thus, the tasks that were selected assess the subject's ability to identify events in sequentially arrayed information and tasks that place a premium on rapid responding. Two of the experimental tasks are presented in a paper-and-pencil format. The first is a Sequence Learning Test modeled after that described by Simon and Kotovsky.[18] In this task, the subject is required to produce the next four characters in a train of sequentially organized characters. Subjects are presented three sequences on a page and allowed 1 minute to complete the page. Four such pages are administered to each subject, with the resulting score based on the number of components in the various sequences correctly produced.

The second paper-and-pencil task is the Symbol Cancelation Task, in which the subject is required to cross out identified target characters in strings of characters. Following instructional materials and practice items, the subject is presented a series of three pages on which 16 lines of 60 characters each are displayed. For each line on the first page there is a different character that the subject must identify and cancel in that line. Each line on pages 2 and 3 has 2 or 4 target characters, respectively. Subjects are allotted 1 minute to complete each page. The score that a subject earns is the number of correctly canceled characters less the omitted characters and the erroneously canceled characters.

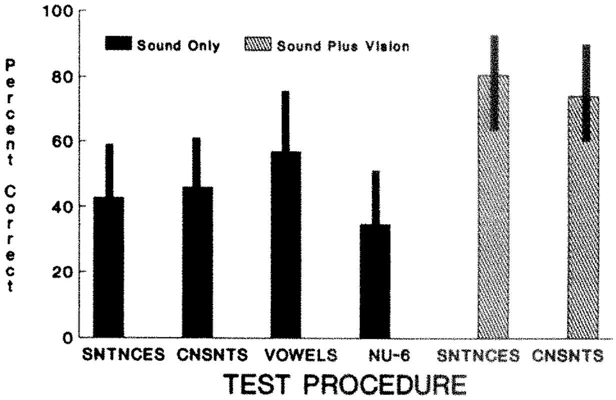
The third experimental cognitive task was the Visual Monitoring Task. This procedure is administered with a microcomputer, a standard computer monitor, and a standard alphanumeric keyboard. The task of the subject is to watch the computer monitor as single-digit numbers are presented one at a time. When the displayed number reflects a pattern of even-odd-even numbers, the subject is to respond on the keyboard. Thus, the subject's task is to maintain the last two digits in working memory and respond when those digits and the currently displayed digit produce the specified pattern. With each digit presentation, the subject must update the two digits in working memory. The numbers are presented at a rate of either one per second or one every 2 seconds, so subjects must respond rapidly. The test procedure lasts 2 minutes and 32 seconds, for each character rate. For each testing rate, the score that a subject receives is a signal detection measure consisting of the standardized probability of a correct response minus the standardized probability of false alarms.[19]

The Raven's Progressive Matrices were presented following the standard administration procedure.[10] The actual set of matrices presented to a subject is based on the subject's response to the first set (Advanced Raven's I). For subjects who perform below the criterion (seven correct) for Advanced Matrices II, the Standard Matrices (A, B, C, D, and E) are administered. For subjects who exceed the criterion, the Advanced Matrices II are administered. The score used for the former subjects is the algebraic combination of the successful matrices completed from the series administered, while the score of the latter subjects consists of the sum of the correct matrices from the advanced set plus 24. Subjects are allowed 40 minutes to complete the Standard Matrices or the Advanced Matrices II.

Audiological Measures. The audiological measures were selected to assess several different capabilities of implant recipients. All of the audiological tests were administered in a sound field at 65 dB sound pressure level within a sound-attenuated chamber by means of a computer-controlled laser videodisc system. Tests administered in a sound-only format included the Consonants Confusion Test (Consonants) and the Vowel Test (Vowels),[15] Phoneme Identification from the NU-6 Word List (NU-6), and the Sentences Without Context Task (Sentences) from the Iowa Phoneme and Sentence Test.[15] The Consonants and the Sentences tests were also administered in a sound-plus-vision format using a videomonitor and the videodisc system.

RESULTS

According to the full complement of tests administered and the self-reports of the implant recipients, all subjects experienced improvement in their ability to respond to and identify acoustic events. As shown in the Figure, however, there was a broad range of audiological outcomes after 18 months of experience with the cochlear implant. A comparison of the groups receiving the two different implants indicated that there were no statistically significant differences between the Nucleus and Ineraid implants on these audiological outcome measures (all F values <2.0). Thus, the audiological data were aggregated across devices for all analyses



Mean percentage of audiological test items correctly identified by 29 consecutively implanted multichannel cochlear implant recipients tested after 18 months of experience with implant. Error bars reflect 1 SD from mean.

TABLE 1. CORRELATIONS BETWEEN SOUND-ONLY AUDIOLOGICAL MEASURES AND PSYCHOLOGICAL PREDICTORS

Audiological	*Visual Monitoring Task-2*	*Visual Monitoring Task-1*	*Sequence Learning Test*	*Symbol Cancelation Task*	*Raven's Progressive Matrices*	*Health Opinion Survey*
Sentences*	.41†	.33*	.32*	.27	.21	.44*
Consonants	.42†	.38*	.47†	.31	.24	.31
Vowels	.51§	.38*	.55§	.42†	.24	.20
Phonemes in words†	.42†	.33*	.39†	.27	.28	.52*

*p<.05.
†p<.01.
§p<.001.

concerning the predictive utility of the psychological variables.

Table 1 shows the Pearson product-moment correlations between the psychological predictor variables obtained prior to operation and the 18-month audiological outcome measures administered in a sound-only format. The Visual Monitoring Task correlated significantly with each of the four sound-only audiological measures, with the character presentation rate influencing the strength of the correlations. That is, the Visual Monitoring Task entailing a character presentation every 2 seconds was associated with correlations accounting for approximately 17% to 26% of the variance in audiological outcome, while the Visual Monitoring Task with one character per second accounted for approximately 11% to 14% of the variance in audiological outcome. Since many implant candidates found the one-per-second rate extremely challenging, it is not surprising that it was less effective as a predictor than the slower rate.

The Sequence Learning Task also correlated significantly with the four audiological measures. Paralleling the Visual Monitoring Task, the Sequence Learning Task accounted for approximately 10% to 31% of the variance in audiological outcome. In the sound-only format, the other paper-and-pencil measure (Symbol Cancelation) correlated significantly with only the Vowel Test. Correlations between the Symbol Cancelation Task and the other sound-only audiological measures only approached statistical significance. Correlations between the Raven's and the sound-only audiological measures also only approached statistical significance. The Health Opinion Survey correlated significantly with the Sentences, Consonants, and phonemes-in-word recognition measures in the sound-only format. Thus, the overall pattern of univariate correlations suggests that better cognitive processing ability and greater motivation and participatory compliance are associated with better audiological outcomes of cochlear implants.

Since the cognitive measures were all administered in a visual medium, it might be expected that they would more effectively predict audiological outcomes that include visual cues. Table 2 shows the correlations between the psychological predictor measures and the two audiological measures administered in a sound-plus-vision format. Although the correlations are somewhat higher between the psychological predictors and the sound-plus-vision Consonants test, the degree of gain is not significant, with the exception of the Raven's. The correlation between the Raven's and the sound-plus-vision Consonants test is the only statistically significant correlation between the Raven's and an audiological outcome. With the exception of the Symbol Cancelation Task, the correlations between predictor measures and the sound-plus-vision Sentences test did not evidence significant improvement relative to the sound-only measure.

Because IQ scores were available from each of these subjects and because intellectual assessments are often included among selection criteria in cochlear implant programs, the Verbal, Performance, and Full Scale WAIS-R IQ scores were correlated with the six audiological measures. These correlations ranged from −.14 to .20, and none approached statistical significance. These results suggest that standardized measures of general intellectual ability are not useful predictors of the audiological outcome of cochlear implants.

The univariate correlations suggest that both cognitive measures and medical compliance and participation can account for significant amounts of variance in the audiological outcome of cochlear implants. To assess whether a combination of medical

TABLE 2. CORRELATIONS BETWEEN SOUND-PLUS-VISION AUDIOLOGICAL MEASURES AND PSYCHOLOGICAL PREDICTORS

Audiological	*Visual Monitoring Task-2*	*Visual Monitoring Task-1*	*Sequence Learning Test*	*Symbol Cancelation Task*	*Raven's Progressive Matrices*	*Health Opinion Survey*
Sentences	.40*	.29	.44†	.39*	.23	.39*
Consonants	.57§	.49†	.58§	.34*	.44†	.36*

*p<.05.
†p<.01.
§p<.001.

TABLE 3. MULTIPLE CORRELATIONS BETWEEN VISUAL MONITORING TASK-2 AND HEALTH OPINION SURVEY AND AUDIOLOGICAL MEASURES

Sound Only				*Sound Plus Vision*	
Sentences	*Consonants*	*Vowels*	*NU-6 Phonemes*	*Sentences*	*Consonants*
.51*	.43*	.47*	.58†	.56†	.60†

*p<.01.
†p<.001.

compliance and cognitive processing would enhance the predictability of cochlear implant outcome, a series of multiple regression analyses were conducted in which the Health Opinion Survey was combined with one of the cognitive measures in a multiple regression to predict the audiological outcome measures. The relatively small number of subjects and the risk of type I errors precludes conducting multiple correlations involving all of the possible variable combinations. Because the univariate analyses suggest that the slower Visual Monitoring Task-2 may be the single best cognitive measure overall, it was selected as the single cognitive measure. These multiple correlations of the combined psychological variables and the six audiological outcome measures are shown in Table 3; the combination of the Health Opinion Survey and the Visual Monitoring Task-2 yields correlations that account for 18% to 36% of the variance in audiological outcome. Although there is some statistical advantage of the combination of the cognitive and compliance measures, the degree of gain relative to the univariate correlations is modest.

DISCUSSION

Although the present study has to be considered a preliminary analysis, the data strongly support the possibility that psychological variables may play a role in the audiological outcome of cochlear implants. First, the significant correlations between the experimental cognitive measures and audiological outcomes suggest that the ability to extract information from sequentially arrayed signals and to rapidly process that information may be relevant to implant success. If the task confronting implant recipients learning to use the device requires the extraction of important acoustic information from an array of ambiguous or new acoustic events, it is reasonable that the cognitive measures included in the present study emerged as effective predictors of implant outcome. Because there was little existing research on which the present work could be based, it was unclear at the inception whether measures based on a visual modality would be useful in predicting a skill in a different sensory modality. In that frankly experimental context, the present results do suggest that cross-modality predictions can be made.

The complete failure of the Raven's and the WAIS-R to predict audiological outcome underscores the unimportance of general intelligence as a factor in cochlear implant outcome. Although the Raven's was not included as a measure of intelligence, evidence[11] does suggest it is a measure of analytical intelligence. Thus, the absence of a correlation between it and the audiological measures is consistent with the absence of correlations involving other measures of intelligence. Although it was the feature extraction and the sequential information that caused the Raven's to be included, the failure of its effectiveness relative to the related experimental measures may be due to the role of general intelligence in Raven's performance. Another difference between the Raven's and the other cognitive measures is the minimal requirement to respond rapidly on the Raven's and the premium placed on rapid responding on the experimental cognitive measures. The general pattern of the results suggests that rapidity in accurate responding might be a critically important feature of cognitive measures that successfully predict implant outcome. Although the experimental cognitive measures have a memory component, the data do not clearly delineate the role that memory may play in predicting outcome. Certainly, future research in this area might profitably assess memory as a factor in implant outcome.

The comparable utility of the Health Opinion Survey also suggests that the level of engagement and participation may be important in implant outcome. Since the experimental cognitive measures are relatively unrelated to intelligence, but they are related to each other, there is some redundant information provided by the repeated univariate correlations. The added predictive utility associated with the combination of the Health Opinion Survey with a cognitive measure suggests that both the attributes of cognitive ability and participatory compliance may be important as implant programs attempt to provide guidance and predict likely outcomes for implant candidates.

Although the results of the present study do not provide sufficient predictive power to select or rule out implant candidates on the basis of psychological measures, the data do suggest that psychological factors need to be considered as part of the implant screening protocol because they may be helpful in informing implant candidates about the range of outcomes they are likely to achieve.

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NINTH SHAMBAUGH/SHEA WEEKEND OF OTOLOGY

The Ninth Shambaugh/Shea Weekend of Otology will be held March 6-8, 1992, at the Chicago Marriott Downtown in Chicago, Illinois. For further information, contact John J. Shea, MD, 6133 Poplar Pike, Memphis, TN 38119; Phone: (901) 761-9720; Fax: (901) 683-8440.

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PROSPECTIVE RANDOMIZED CLINICAL TRIAL OF ADVANCED COCHLEAR IMPLANTS: PRELIMINARY RESULTS OF A DEPARTMENT OF VETERANS AFFAIRS COOPERATIVE STUDY

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This study is a randomized clinical trial to compare the efficacy and safety of one single-channel implant (the 3M/Vienna intracochlear device) and two multichannel cochlear implants (the Nucleus 22-channel device and the Smith & Nephew Richards Ineraid device). Seven Veterans Administration centers are participating in the study. The patient population consists of 82 veterans with postlingual, bilateral profound sensorineural hearing losses who obtain no benefit from amplification. All potential subjects were screened with a battery of audiologic tests. The three device groups are balanced on baseline patient characteristics. One month after operation, the devices were stimulated and programmed and the patients evaluated. Follow-up evaluations were completed at 3 and 12 months poststimulation and then annually until the end of the study. The 12-month data indicate that performance with the multichannel devices is superior to that with the single-channel device.

KEY WORDS — clinical trial, cochlear implant, comparative study.

Cochlear implants have been in a state of development for approximately 20 years, initially in a single-channel format and then in several multichannel formats. Fundamentally, these devices attempt to address the problem of the profoundly hearing-impaired person who is unable to derive any benefit from conventional amplification. A single-channel prosthesis is easier to fabricate, and a short electrode that does not go deep into the cochlea can be used with it. A multichannel prosthesis has a more complex pattern of stimulation and uses an electrode array that is more invasive to the cochlea.

Several studies have provided direct comparisons between single-channel and multichannel electrical stimulation. Although multichannel stimulation has, in the direct comparison studies, been found to provide greater improvements in speech recognition ability than single-channel stimulation, large individual differences were noted and many issues required further exploration.[1-4] To date, there has been no randomized prospective study of various cochlear implants.

The purpose of this research was to do a prospective randomized study comparing the efficacy of several advanced cochlear implants, all of which had been shown to produce various amounts of open-set speech recognition.[5-8] A secondary goal was to examine the predictive value of preoperative audiometric, radiographic, psychological, and electrical tests on postoperative results. Other objectives included the monitoring of patients for a period of up to 5 years to measure the effects of chronic stimulation and studying the long-term effects of implantation on the histologic characteristics of the temporal bone.

This paper is a preliminary report focusing on the results obtained 1 year poststimulation.

METHODS AND MATERIALS

Eighty-four (82 men, 2 women) postlingually profoundly deafened adult veterans who served in the United States Armed Forces, ages 30 to 80 years (mean, 59.3 years), were randomized to one of three cochlear prostheses (the Nucleus 22-channel cochlear implant, the Ineraid 4-channel device, or the 3M/Vienna single-channel implant) at seven Veterans Administration medical centers. The UCSF/Storz device, which in its 4-channel form was reported to provide open-set speech recognition to patients, was included in the original protocol but was taken off the market prior to the beginning of the study. The mean length of profound deafness was 15 years, with a range of 1 to 45 years. The participating Veterans Administration centers are Ann Arbor, Mich; Houston, Tex; Iowa City, Iowa; Long Beach, Calif; Manhattan, NY; Seattle, Wash; and West Haven, Conn.

All patients met these criteria for inclusion: age 18 years or over, bilateral profound sensorineural hearing loss with no benefit from appropriate am-

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TABLE 1. PROTOCOL FLOWCHART

1. Identification of protocol candidate
2. Initial interview
   Initial hearing evaluation → patient exclusion
3. Otologic examination
   Radiographic studies
   Medical evaluation and patient history → patient exclusion
   Initial hearing aid evaluations
4. Iowa tests
   MAC tests
   SPAC tests
   Speech tracking
   Brain stem auditory evoked response testing → patient exclusion
5. Blood tests
   Electronystagmography
   Tinnitus questionnaire (for bothersome tinnitus only)
   Quality of life questionnaires
   Performance inventory for profound hearing loss
   Psychological evaluation → patient exclusion
6. Approval by associate chairman for audiology → patient exclusion
7. Ear selection
   Informed consent
   Round window stimulation
8. Randomization
9. Surgery (order external device components)
10. Device stimulation
    Rehabilitation
    Request for donorship of temporal bones
11. 3-month poststimulation follow-up visit
12. 12-month poststimulation follow-up visit
13. 24-month poststimulation follow-up visit
14. Annual follow-up visits thereafter

plification (no open-set speech recognition), postlingually deafened, English speaking and literate, absence of complicating medical conditions, psychologically stable, no previous cochlear implant, and agreement to participate in the study after being informed of all aspects of the protocol.

In order to determine patient eligibility, the patient was entered into the protocol shown in Table 1. The auditory test battery used to evaluate patient performance with appropriate amplification preoperatively is shown in Table 2. All tests, except for Speech Tracking, were administered via videotape or audiotape. Examiners were given written instructions as to the preferred method of test administration to ensure uniformity.

The psychological evaluation included the Wechsler Adult Intelligence Scale, The Minnesota Multiphasic Personality Inventory, and the Beck Depression Scale.

The medical evaluation consisted of an assessment of the veteran's general medical history and physical examination, with particular attention paid to the otologic aspects of both. A high-resolution computed tomography scan was performed in order to demonstrate cochlear patency.

Following the evaluation and the determination of candidacy by the investigators at each site, final approval for inclusion into the study was given by the study chairman's office. Eligible patients were then scheduled for round window stimulation. Patients were stratified on the basis of a positive or negative response to electrical stimulation. Testing included electrical threshold, dynamic range, gap detection, adaptation, and temporal difference limen. All patients were tested with the Nucleus stimulator.

The following guidelines were used for ear selection: 1) the poorer hearing ear, 2) if either ear was fitted with an amplification device to obtain environmental cues, the contralateral ear was implanted, 3) if one scala tympani appeared on computed tomography scan to have a more normal structure than the other, the more normal one was selected,

TABLE 2. AUDIOLOGIC TEST BATTERY

Iowa Sentence Without Context Sound Only
Iowa Open-Set Everyday Sound Recognition
Iowa Medial Consonant Test Sound Only
Iowa Medial Consonant Test Sound and Vision
Iowa Medial Consonant Test Vision Only
Iowa Male/Female Recognition
MAC Question/Statement
MAC Noise/Voice
MAC Accent
MAC CID Everyday Sentences
MAC Monosyllabic Words (NU-6)
MAC Four Choice Spondee
Iowa Sentence Without Context Sound and Vision
SPAC Roving Stress
SPAC Rise-Fall
SPAC Vowel Height and Place
SPAC Initial Consonant Voicing and Manner
SPAC Final Consonant Voicing and Manner
SPAC Initial and Final Consonant Place of Articulation
SPAC Phoneme Recognition
Iowa Spondee Recognition in Noise
Iowa Sentence Without Context Vision Only
Speech Tracking

4) the ear with absent caloric response, and 5) chronic ear disease in one ear. All things being equal, the patient's preference was the determining factor.

The time interval between round window stimulation and randomization to a device was approximately 1 month. A stratified randomization with permuted blocks was used for this study. Stratification was based on participating institution and positive or negative results on round window stimulation. Each patient was randomized to a primary device and a secondary single-channel device. The secondary device was either the 3M/Vienna or the Nucleus single-channel prosthesis and was to be implanted when fewer than 50% of the electrodes of either of the multichannel devices could be inserted because of pathologic changes within the scala tympani encountered during operation. The operation was completed within 5 working days of randomization. The study chairman participated in the first operation for each device at most centers.

TABLE 3. FOLLOW-UP EVALUATION

Otologic examination
Electrical threshold and comfort levels determined for each electrode of implanted device
Sound-field pure tone thresholds
Speech detection thresholds
Speech reception thresholds
Speech discrimination scores
Most comfortable loudness level
Uncomfortable loudness level
Iowa battery
Minimum Auditory Capabilities test
Speech pattern contrast test
Speech Tracking
Quality of life questionnaires (patient and index relative)
Performance inventory for profound hearing loss
Tinnitus handicap questionnnaire (only for patients having bothersome tinnitus at screening)

TABLE 4. AUDIOLOGIC CATEGORIES

Category 1	Prosodic characteristics
	MAC Accent
	MAC Noise/Voice
	SPAC Rise/Fall
	Iowa Male/Female Recognition
	MAC Question/Statement
	SPAC Roving Stress
Category 2	Lipreading enhancement
	Speech Tracking (sound and vision)
	Iowa Medial Consonant Sound and Vision
	Iowa Sentence Without Context Sound and Vision
Category 3	Phonetic level
	SPAC Initial Consonant Manner
	SPAC Final Consonant Voicing
	Iowa Medial Consonant Sound Only
	SPAC Initial Consonant Voicing
	SPAC Final Consonant Manner
	SPAC Vowel Place
	SPAC Vowel Height
	SPAC Initial Consonant Place of Articulation
	SPAC Final Place of Articulation
Category 4	Spondee tests
	MAC Four Choice Spondee
	Iowa Spondee Recognition in Noise
Category 5	Open set discrimination
	MAC CID Everyday Sentences
	Speech Tracking (sound only)
	Iowa Sentence Without Context Sound Only
	Speech discrimination (W-22)
	SPAC Phoneme Recognition
	NU-6 words

Patients were stimulated approximately 4 weeks postoperatively. Representatives from each of the manufacturers were present at each site during the first stimulation to ensure proper procedure. The audiologic test battery (Table 2) was administered immediately following the completion of the programming of the device. All patients then participated in a 10-session training program consisting of speech tracking. Tracking was done under the three conditions, 10 minutes each, of lipreading alone, lipreading plus implant, and implant alone.

Follow-up evaluations, outlined in Table 3, were scheduled for 3 months and 1, 2, 3, 4, and 5 years poststimulation. The same methodology used to administer the tests preoperatively was used for the follow-up evaluations. All tests were administered in the same order at all sites and different randomizations of tests were used for follow-up evaluations. The randomizations were outlined in the protocol and uniform across all centers. No study patient underwent additional audiologic testing or rehabilitation for the duration of the study.

The sample size in this study for the four-treatment-group design allows for the detection of a 50% difference in means of the lowest and highest groups for the majority of the audiologic tests with a two-sided alpha, adjusted for multiple comparisons, of 0.05 and a power of 0.80. In addition, a composite

TABLE 5. CAUSE OF DEAFNESS

Cause	Device Used (No. of Patients) Multichannel	Single-Channel
Noise exposure	27	8
Unknown	24	8
Ototoxicity	11	2
Head trauma	10	4
Meningitis	9	5
Otosclerosis	9	3
Infection	7	2
Hereditary (progressive)	6	1
Mumps	2	0
Measles	2	0
Lupus	1	0
Other	6	4

index, a summary measure of audiologic tests used in this study, was developed by using principal components analysis for each of the test groups in Table 4 and all of the tests combined.[9] The categorizing of the audiologic battery was an attempt to group together tests that evaluate similar functions to enable us to obtain one composite score for each function. It was estimated that an 18% change or greater in the overall composite score among treatment groups would be detectable with a power of 0.80. Powers for analyses comparing the multichannel devices to the single-channel device will be higher, because only two groups are compared and the sample size is larger for the combined group. Analyses comparing the two multichannel devices to the single-channel device were conducted by using Student's *t* test on the change scores from baseline to 1 year. At baseline, the two groups were well balanced relative to patient and audiologic characteristics. At this preliminary stage of the study, only the single-channel versus multichannel question could be answered. The 2-year poststimulation test results will be used as the final outcome data to compare the three devices to each other.

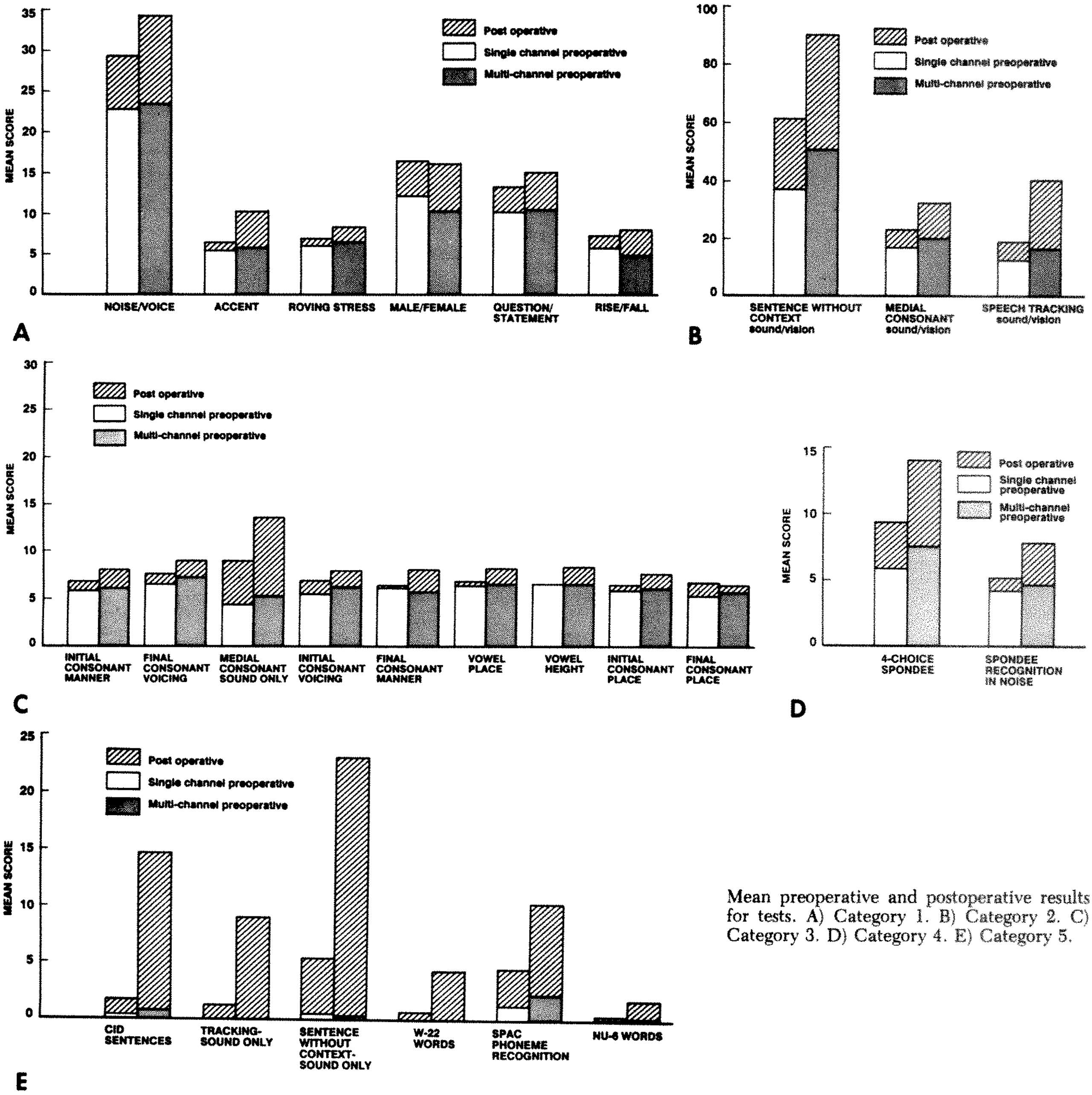
RESULTS

Eighty-two patients were implanted. Twenty had been randomized to and implanted with the 3M/Vienna single-channel device. Randomization to the 3M/Vienna device was halted at 20 subjects, because the 3M Company made a corporate decision to discontinue manufacturing the prosthesis. Thirty patients have been randomized to and implanted with the Nucleus multichannel device and 30 patients have received the Ineraid prosthesis. Two additional patients were randomized to a multichannel device but because of an obstruction in the cochlea detected during operation received single-channel devices. (One received a 3M/Vienna device and the other a Nucleus single-channel device.) For statistical reasons, these patients were included in the treatment group to which they were randomized for all analyses.

Of the 62 patients randomized to the multichannel devices, 60 were men and 2 were women. All 20 patients randomized to the 3M/Vienna device were men. The mean age at time of implantation was 59.9 years, with a mean length of profound deafness of 15.3 years for the multichannel group. The mean age at time of implantation was 57.6 years for the single-channel patients, whose mean length of profound deafness was 14.3 years. The causes of deafness for both groups are shown in Table 5. When multiple causes of deafness were given for an individual, the patient was listed under each of the categories. One-year poststimulation data were compared to preoperative scores on 45 patients with multichannel devices and 17 3M/Vienna users. The remainder of the recipients of the Nucleus and Ineraid devices have not reached the 1-year evaluation point. Of the 3 remaining 3M/Vienna users, 1 is deceased, 1 did not receive auditory sensation postoperatively, and the third required removal of the de-

TABLE 6. AUDIOLOGIC CATEGORY RESULTS

	Multichannel Device Mean	SD	Single-Channel Device Mean	SD	p
Composite index					
Preoperative	10.3	4.2	9.4	2.8	
1 y postoperative	42.2	36.4	17.9	12.1	.0001
Category 1 index					
Preoperative	26.8	7.0	26.4	5.9	
1 y postoperative	38.8	8.2	33.5	4.6	.002
Category 2 index					
Preoperative	8.6	20.3	−0.1	8.9	
1 y postoperative	37.1	31.9	18.4	18.9	.006
Category 3 index					
Preoperative	12.0	4.3	11.4	3.8	
1 y postoperative	21.0	8.4	16.3	6.0	.004
Category 4 index					
Preoperative	8.9	4.8	7.2	4.1	
1 y postoperative	16.0	5.7	11.2	3.7	.002
Category 5 index					
Preoperative	1.1	2.0	0.8	1.4	
1 y postoperative	29.2	34.9	6.4	10.4	.0001



Mean preoperative and postoperative results for tests. A) Category 1. B) Category 2. C) Category 3. D) Category 4. E) Category 5.

vice because of severe facial stimulation upon activation. No patients have been lost to follow-up.

Results demonstrated that patients who received the multichannel devices showed significantly greater improvement in their auditory responses postoperatively than those patients who were implanted with the 3M/Vienna single-channel device. As shown in Table 6, differences as measured by the composite index were highly significant overall ($p < .0001$) as well as for each of the categories of audiological tests.

Three of the six tests in category 1 (prosodic characteristics) showed significant differences between device groups: MAC Noise/Voice test ($p = .0054$), MAC Accent ($p = .0001$), and SPAC Roving Stress ($p = .0369$; see Figure, A).

In category 2, lipreading enhancement, one of the three tests, Speech Tracking (sound and vision), showed a significant difference between the multichannel group and the single-channel group ($p = .0001$; see Figure, B).

The Figure (C) shows the results for the phonetic level (category 3). Five of the nine tests showed a significant difference: SPAC Vowel Height ($p = .0011$), SPAC Vowel Place ($p = .0445$), SPAC Final Consonant Voicing ($p = .0352$), SPAC Final Consonant Manner ($p = .0089$), and SPAC Initial Consonant Place of Articulation ($p = .0462$).

Results from both of the spondee tests included in

TABLE 7. TESTS SHOWING SIGNIFICANT ($p < .05$) IMPROVEMENT WITH 3M/VIENNA DEVICE

Category 1	Iowa Male/Female Recognition MAC Question/Statement MAC Noise/Voice SPAC Roving Stress
Category 2	Iowa Medial Consonant Test Sound and Vision Iowa Sentence Without Context Sound and Vision Speech Tracking (sound and vision)
Category 3	Iowa Medial Consonant Test Sound Only
Category 4	MAC Four Choice Spondee Iowa Spondee Recognition in Noise
Category 5	Iowa Sentence Without Context Sound Only SPAC Phoneme Recognition

category 4 differed significantly: Iowa Spondee Recognition in Noise ($p = .0026$) and MAC Four Choice Spondee ($p = .0015$; see Figure, D).

Performance differences were found on all of the open-set discrimination tests (audition alone) listed in category 5: W-22 words ($p = .0025$), Iowa Sentence Without Context Sound Only ($p = .0011$), MAC CID Everyday Sentences ($p = .0001$), NU-6 words ($p = .0020$), SPAC Phoneme Recognition ($p = .0004$), and Speech Tracking ($p = .0002$; see Figure, E).

Significant improvement ($p < .05$) was noted for 12 of 26 audiologic tests when the preoperative data were compared to the postoperative results for the 3M/Vienna device. Four of the tests were in category 1, 3 in category 2, 1 in category 3, 2 in category 4, and 2 in category 5 (Table 7). Patients implanted with the multichannel devices, however, showed significant improvement ($p < .05$) on all of the tests in all categories when preoperative results were compared to the 1-year data. Both groups showed significant improvement on the Iowa Open-Set Everyday Sound Recognition test.

DISCUSSION

The preliminary results of this study demonstrate the superiority of two multichannel cochlear implants over the single-channel system despite previously published reports of excellent results with the Vienna single-channel device.[4-7] Our results are more in agreement with those obtained by Gantz et al.[2]

Patient performance using the 3M/Vienna device improved on 12 of the 26 tests listed in the audiologic categories from time of randomization to the 1-year data collection point. Combined scores for the same period for patients using the Ineraid and Nucleus multichannel devices improved significantly on all 26 tests. In addition, for 7 of the 12 tests on which the 3M/Vienna users did improve, the multichannel users improved to a significantly greater degree. There was no test on which the single-channel users performed better than the multichannel users.

It is clear from these data that the multichannel cochlear prostheses provide improved access to both the suprasegmental and segmental aspects of the speech signal as well as to environmental sounds. The patients using the 3M/Vienna device showed improvement in the greatest number of tests in categories 1 and 2, ie, prosodic features and lipreading enhancement. Even so, performance of the multichannel users in these areas was either equal to or better than the single-channel results.

During the planning of this study, we had hoped that the results of this study would define auditory conditions that might be better served by a single-channel implant. To date, we have been unable to identify any areas that would dictate the implantation of a single-channel device over a multichannel system.

Since our final analysis regarding the differences between devices will be based on data collected 2 years poststimulation, we are continuing to evaluate all of the patients according to protocol.

We conclude that the multichannel cochlear implants used in this study provide significantly greater auditory benefits than the 3M/Vienna single-channel device.

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BILATERAL ACOUSTIC NEUROFIBROMATOSIS (NEUROFIBROMATOSIS 2): A DISORDER DISTINCT FROM VON RECKLINGHAUSEN'S NEUROFIBROMATOSIS (NEUROFIBROMATOSIS 1)

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A case report of bilateral acoustic neurofibromatosis (NF-2) is presented with temporal bone histopathology. The distinct clinical and genetic features of NF-2 are reviewed in the context of recent data that show it to be a disorder distinct from classic von Recklinghausen's disease (NF-1).

KEY WORDS — bilateral acoustic neurofibromatosis, temporal bone, von Recklinghausen's disease.

INTRODUCTION

Acoustic neuroma (eighth nerve schwannoma) may present as a sporadic lesion or as one manifestation of a genetically determined syndrome. (The histologically accurate term "eighth nerve schwannoma" will be used in lieu of "acoustic neuroma.") It has recently become clear that central neurofibromatosis (NF-2) is a disorder distinct from classic von Recklinghausen's disease (NF-1). In the central form of neurofibromatosis (NF-2), bilateral eighth nerve schwannoma is common, whereas in von Recklinghausen's neurofibromatosis (NF-1), eighth nerve schwannoma is rare.[1] The clinical features and genetics of NF-2 are illustrated by a case report with temporal bone histopathology. The clinical features that distinguish NF-1 from NF-2 and sporadic solitary eighth nerve schwannoma will be reviewed.

CASE REPORT

This 20-year-old man was first evaluated in 1979 for hearing loss and tinnitus in the left ear. There was no history of vertigo. Neurofibromatosis had been previously diagnosed on the basis of resection of a neurofibroma from the right brachial plexus at age 9, and a cervical laminectomy at age 13 for schwannomas of the spinal cord. He was otherwise in good health. Physical examination revealed no abnormalities of the head and neck other than a hearing loss. There were no café au lait spots detected, although a Woods lamp was not used. Ophthalmologic evaluation demonstrated amblyopia, no Lisch nodules, and a decreased right corneal reflex.

An audiogram showed a left high-frequency sensorineural hearing loss (Fig 1), and an electronystagmogram showed an absent caloric response in the left ear. A computed tomography (CT) scan of the head (Fig 2) showed a 2.5-cm mass in the left cerebellopontine angle (CPA), and a large, heavily calcified mass was seen in the right CPA.

The patient underwent a left suboccipital craniectomy with drilling out of the internal auditory canal and subtotal removal of the eighth nerve schwannoma, with preservation of hearing. Within months, the patient developed weakness of the right hand, and at 21 years of age, he underwent a laminectomy of the C-5 through C-7 vertebrae and removal of a schwannoma of the spinal cord. Because of progressive weakness of the left hand, at the age of 22, a T-7 through T-10 laminectomy and biopsy of an intramedullary spinal cord astrocytoma was performed. This was treated postoperatively with 50 Gy of radiation. In the following months, the patient had deterioration of hearing on the right and dysphagia with aspiration.

At 24 years of age, the patient underwent a right suboccipital craniectomy and drilling out of the internal auditory canal, with total macroscopic removal of an eighth nerve schwannoma and subtotal removal of a meningioma that was also located in the right CPA. Hearing was not preserved. In the months following this procedure he developed weakness of the lower extremities and underwent an additional exploration of the cervical spine with removal of an intraspinal schwannoma of the spinal root. Hydrocephalus developed in the immediate postoperative period for which a ventriculoperito-

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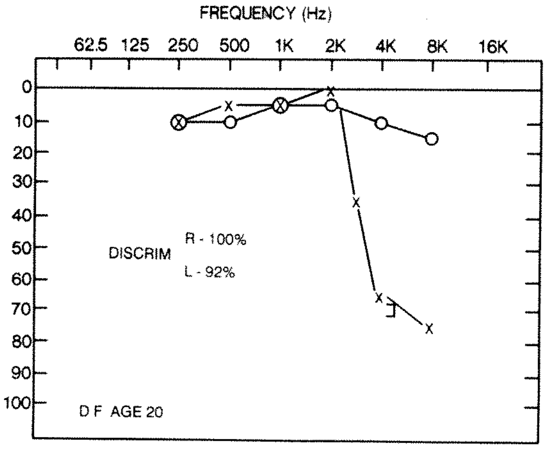

Fig 1. Audiogram of patient at presentation showing left ear high-frequency sensorineural hearing loss.

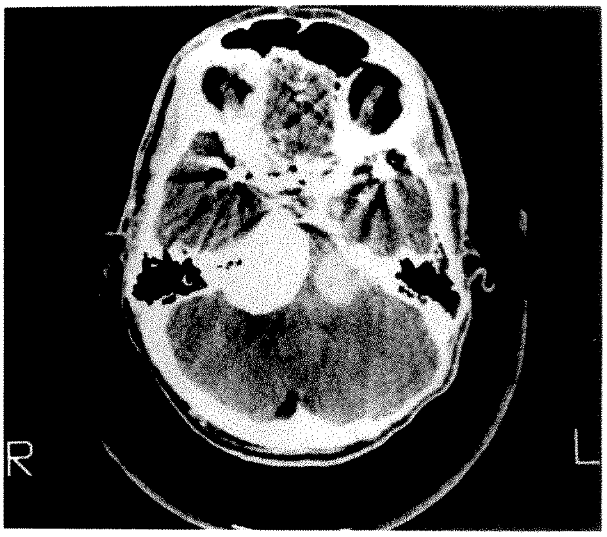

Fig 2. Axial computed tomogram through petrous apices showing large calcified mass at right cerebellopontine angle (CPA) and 2.5-cm mass at left CPA.

neal shunt was performed 1 month later. Because of continued neurologic problems from brain stem compression, a second, left occipital craniectomy and macroscopic total removal of residual eighth nerve schwannoma was performed. Hearing was not preserved. Postoperatively, he experienced progressive respiratory distress due to tracheal and spinal cord compression from cervical masses, and died of respiratory failure.

TEMPORAL BONE FINDINGS

Right Ear. The most striking finding is a large tumor having the histologic features of schwannoma and filling the internal auditory canal with circumferential erosion of bone (Fig 3A). Corpora amylacea are found in the dense tumor capsule. Surgical defects are present in the internal auditory canal and the middle portion of the endolymphatic sac. These areas contain healthy-appearing adipose tissue grafts. The cochlear nerve is nearly totally destroyed by tumor, with only a few spiral ganglion cells remaining. The facial nerve is displaced anteriorly and flattened by the tumor. The geniculate ganglion is invaded by tumor. About 75% of the facial nerve fibers remain in the tympanic and mastoid segments. The center of the nerve trunk contains a small accumulation of cells that appears to represent a separate schwannoma arising within the facial nerve. The tumor has extended to the fundus of the internal auditory canal and has entered the cribrose area of the superior and inferior divisions of the vestibular nerve, which are severely degenerated. The tumor also has extended into the basal regions of the superior crista and the utricular macula. There is severe hydrops of the saccule; however, the macula of the saccule appears normal.

There is moderate hydrops of all turns of the cochlear duct. Tumor is seen in the scala tympani of the basal turn (Fig 3B). A site of invasion of the cochlea cannot be found, and it is likely that the intracochlear tumor is a separate lesion. The organ of Corti is well preserved. The hair cell population is decreased in the basal turn but normal in the middle and apical turns. In both the cochlear and vestibular labyrinth, the perilymph and endolymph contain acidophilic fluid indicative of increased protein content.

Left Ear. The internal auditory canal is widely flared by a large lobulated tumor (Fig 3C). Characteristic of a schwannoma, there are areas of cellular palisading. Near the fundus of the auditory canal, the tumor is less cellular and appears slightly myxomatous. The tumor extends into the superior part of the vestibule and ampullae of the superior and lateral semicircular canals. There is a thick layer of fibrous tissue lining the posterior wall of the petrous bone posterior to the internal auditory canal.

There is moderately severe degeneration of the membranous labyrinth. The organ of Corti is severely degenerated, with no hair cells remaining. Only a small area of stria vascularis can be seen in the basal turn. There is fibrous tissue in the scala vestibuli of the basal turn. Reissner's membrane is ruptured. There is no tumor invasion into the cochlea. Both the perilymphatic and endolymphatic spaces contain acidophilic fluid. The spiral ligament and tectorial membrane are severely degenerated in all turns. The cochlear neurons and spiral ganglion cells are almost completely missing, with only a few scattered cell bodies remaining. The vestibular nerves, cristae, and macula of the saccule are degenerated. There is partial degeneration of the macula of the utricle. There is bone and connective tissue in the posterior semicircular canal. The facial nerve appears to be intact in its tympanic and mastoid segments.

DISCUSSION

The case report demonstrates the clinical course

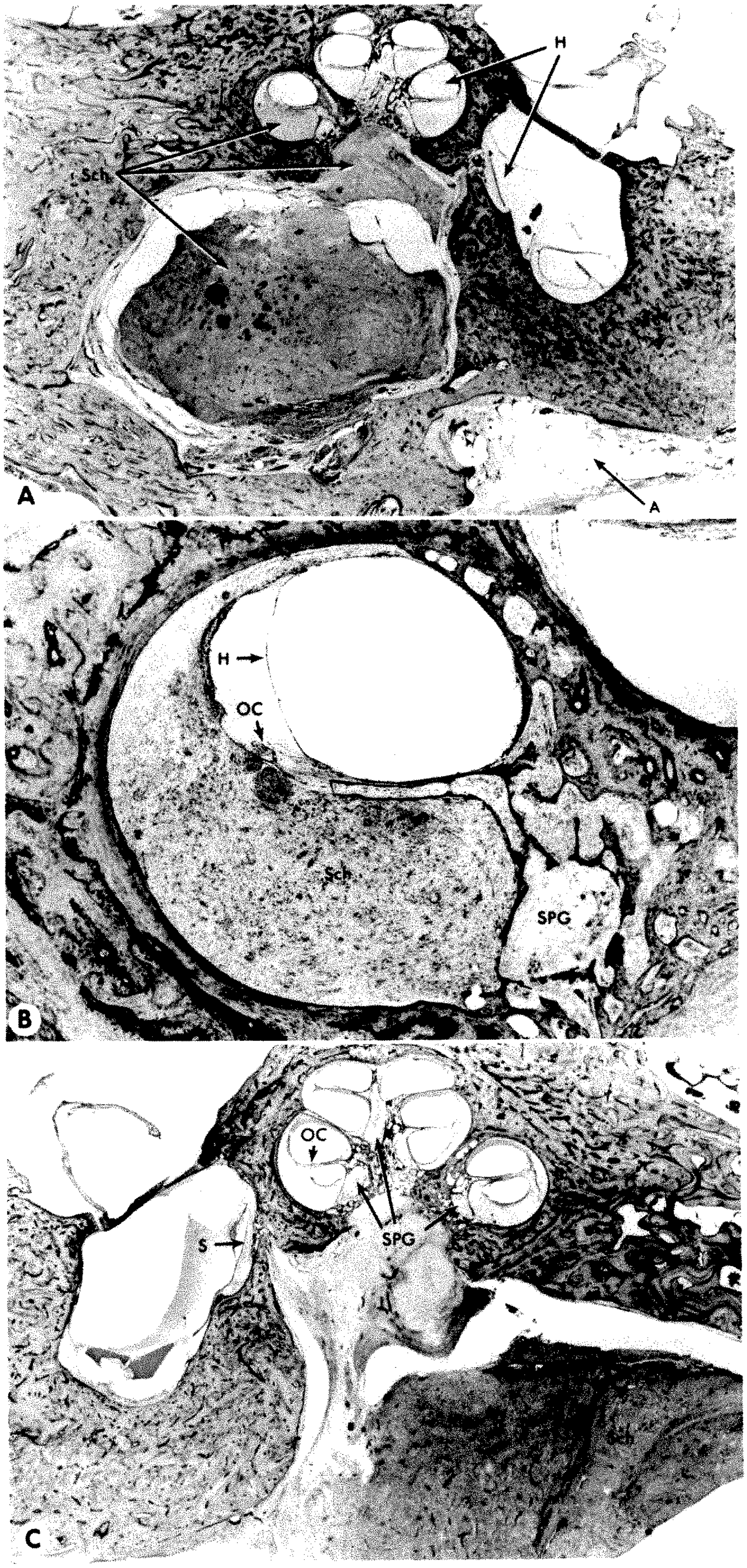

Fig 3. Horizontal sections of temporal bone. A) Section of right temporal bone showing residual schwannoma (Sch) within widened internal auditory canal (H & E, original x8.3). Hydrops (H) of cochlea and saccule is seen. Adipose tissue graft (A) is present on posterior fossa surface of petrous bone. B) Separate focus of Sch is present in scala vestibuli of basal turn of right cochlea (H & E, original x56). There is endolymphatic hydrops (H) and normal organ of Corti (OC) but marked loss of spiral ganglion cells (SPG). Reissner's membrane (R) is ruptured. C) Section of left temporal bone showing Sch of internal auditory canal and cerebellopontine angle (H & E, original x8). There is marked degeneration of neuroepithelium of saccule (S) and OC and marked loss of SPG.

TABLE 1. GENETIC FEATURES

	NF-1	*NF-2*
Incidence	1:3,000	1:100,000
Inheritance	Single gene, autosomal dominant	Single gene, autosomal dominant
Mutation rate	High known	Uncertain
Penetrance	High	High
Variance of expression	High	Low with respect to eighth nerve schwannomas
Locus of defect	Long arm, chromosome 17 (pericentromere region)	Long arm, chromosome 22

TABLE 2. CLINICAL FEATURES

	NF-1	*NF-2*
Six or more café au lait macules	75%	0%
Lisch nodules	95%	0%
Eighth nerve schwannoma	Same as incidence in general population	Approaches 100%
Age at onset of signs	1st decade	2nd or 3rd decade

of a patient with NF-2. Central lesions, including bilateral eighth nerve schwannomas, cranial and spinal meningiomata, and glioma of the brain stem, are commonly seen in NF-2.[1,2] In NF-1, lesions are generally seen in skin, peripheral nerves, and spinal roots, whereas central tumors other than benign optic gliomas are rare. Bilateral eighth nerve schwannomas are not generally seen in NF-1.[3] However, as NF-1 and NF-2 may have both central and peripheral nervous system manifestations, the terms "peripheral neurofibromatosis" and "central neurofibromatosis" have been discarded.[1]

Histology. Although the cytologic pattern seen in bilateral eighth nerve schwannomas of NF-2 cannot be distinguished from that seen in unilateral eighth nerve schwannoma, there are histopathologic differences. Previous temporal bone studies of bilateral eighth nerve schwannomas thought to be a manifestation of von Recklinghausen's disease (but which are undoubtedly cases of NF-2) have demonstrated that the schwannomas of NF-2 are larger in size and often multicentric, in contrast to unilateral lesions; these findings are consistent with those in the case reported here.[4-6] In addition, in NF-2, the internal auditory canal more often shows marked enlargement and bony erosion. In contrast to the well-encapsulated unilateral eighth nerve schwannoma, the lesions of NF-2 are often multilobulated and surround, rather than displace, the facial and cochlear nerves.[7] Total or near-total tumor removal with hearing preservation is much more difficult, even with small tumors.[7]

Genetics. The genetic features of NF-1 and NF-2 are summarized in Table 1. Both NF-2 and NF-1 are autosomal dominant traits. The incidence of NF-2 is believed to be 1:100,000; hence, it is significantly less common than NF-1, which has a frequency of 1:3,000.[8] Both disorders appear to be single gene defects with a high degree of penetrance (90% to 100%).[3] As many as half of the cases of NF-1 are nonfamilial cases believed to be caused by mutation de novo. This implies that the mutation rate at the gene locus responsible for NF-1 is high. The expression of the NF-1 gene is quite variable, with some patients having minimal stigmata while others have hundreds of cutaneous neurofibromata. In contrast, all patients with NF-2 have bilateral eighth nerve schwannomas. However, the presence of other tumors is more variable.[3]

Recent investigations in gene mapping have utilized recombinant DNA techniques to generate large numbers of polymorphic gene markers. By demonstrating cosegregation of a disease trait with a particular DNA marker displaying restriction fragment length polymorphisms, the chromosomal location of the gene causing the disorder can be determined. This method was used by Seizinger et al,[9,10] Rouleau et al,[2] and Wertelecki et al[11] to show the primary inherited defect for NF-2 to be a defect in the long arm of chromosome 22. For NF-1, this technique was used to show that the NF-1 gene is located near the centromere of chromosome 17.[12,13] Thus, despite some similarities between NF-1 and NF-2, these are not allelic mutations at the same locus, but genetic defects at separate loci. It has been speculated that NF-2 may be due to a loss of a suppressor gene. This situation would be similar to what has been found in retinoblastoma and Wilms' tumor, which are brought about by loss of the chromosome regions 13q and 11p, respectively.[2,10]

Clinical. The contrasting clinical features of NF-1 and NF-2 are summarized in Table 2.

Café au lait macules are a common finding in NF-1. The presence of six café au lait macules over 15 mm in diameter (postpubertal) or five macules of 5 mm (prepubertal) is a useful criterion in diagnosing NF-1, as 75% of patients with NF-1 will meet this criterion.[2] By contrast, Martuza and Ojemann[7] found that only 2 of 15 patients with NF-2 had six or more café au lait macules, and Kanter et al[3] found that none of 76 patients with NF-2 had six or more macules. Our patient had no café au lait macules. However, a Woods lamp examination, which is the preferred method for detecting these lesions, was not done.

Lisch nodules, which are pigmented raised hamartomas of the iris, are seen in 95% of postpubertal patients with NF-1. Lisch nodules either do not occur or occur only very rarely in patients with NF-2.[3,7] In contrast, patients with NF-2 less commonly have apicitis or cataracts at an early age.

Patients with NF-1 usually show clinical signs of the disorder in the first decade of life. By contrast, clinical signs or symptoms of NF-2 usually present in the second or third decade of life (mean of 21 years). A maternal effect has been reported in NF-2 whereby patients inheriting the disorder from their mothers have an average age at onset of 18 years, compared to 24 years for patients inheriting the disease from their fathers.[3] Patients presenting with eighth nerve schwannomas in their teens or early twenties also distinguish NF-2 from sporadic unilateral eighth nerve schwannoma in the general population, which usually presents in the fifth or sixth decade of life.[7]

Most patients with NF-2 develop other tumors of the central nervous system in addition to eighth nerve schwannomas, as was demonstrated in the case report. Martuza and Ojemann[7] reported that 75% of patients with NF-2 develop additional central nervous system tumors, most commonly meningiomas. This tendency to form additional tumors is greater in those patients presenting with clinical symptoms before 21 years of age.[7,11]

Cutaneous neurofibromas may be present in both disorders, but in NF-2 the lesions are fewer in number and more difficult to detect.[7]

Osseous malformation (eg, kyphoscoliosis), macrocephaly, sphenoid wing dysplasia, speech impediments, low IQ, impaired fine motor skills, and seizure disorders have all been reported to be associated with NF-1, but are not common with NF-2.[9]

Because the central nervous system tumors of NF-2 often lead to deafness, balance disorders, and paralysis, the overall neurologic morbidity may be greater than in NF-1. However, one of the most devastating sequelae that can occur with NF-1 is malignant transformation of a neurofibroma to sarcoma, which occurs in up to 5% of cases. Malignant transformation is not common in tumors associated with NF-2.[3,14]

Other than bilaterality, the presenting symptoms of eighth nerve schwannomas in NF-2 are similar to those seen in patients with spontaneous unilateral eighth nerve schwannomas. Of the 74 patients with NF-2 reviewed by Kanter et al,[3] 50% presented with bilateral hearing loss (whether this was symmetrical or asymmetrical bilateral hearing loss was not recorded), 21% with unilateral hearing loss, 10% with imbalance, 8% with tinnitus, 5% with headache, and 6% with other symptoms.[3]

Current accepted criteria for the diagnosis of NF-2 are 1) evidence of bilateral internal auditory canal masses by CT or magnetic resonance imaging (MRI); or 2) one of the following in a patient whose parent, sibling, or child has documented NF-2: unilateral CT or MRI evidence of an internal auditory mass, or a meningioma, glioma, ependymoma, or Schwann cell tumor.[2]

Neurofibromatosis 2 should be suspected in a person with an apparent unilateral eighth nerve schwannoma with onset of symptoms before age 30; a child with a meningeal or Schwann cell tumor; a person with no family history of NF-1 who has one or more neurofibromas but few, or no, café au lait macules and no Lisch nodules; or a close relative of a person with known NF-2. Currently, the best initial screening test for such patients is a high-quality gadolinium-enhanced MRI scan. Once the diagnosis is made, the patient and relatives at risk need to be evaluated and counseled by a clinical team experienced in the management of the many problems associated with NF-2.

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IMPLICATION OF SURFACTANT APOPROTEIN IN OTITIS MEDIA WITH EFFUSION

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A two-site simultaneous immunoassay using monoclonal antibodies against human surfactant apoprotein (SAP) was used to measure SAP in middle ear effusions (MEEs). In 130 MEE samples from children with otitis media with effusion, SAP was detected in 54 samples (SAP-positive cases, 41.5%). In the remainder, the SAP concentration was below the sensitivity of the immunoassay (SAP-negative cases, 58.5%). A significant difference in periods of observation was found between the SAP-positive cases (17.3 ± 16.8 months) and the SAP-negative cases (26.2 ± 22.5 months) ($p < .01$). The percentage of positive cases was highest in the serous MEE group (81.2%) and decreased in the purulent MEE group (57%), the mucoid MEE group (30%), and the hyperviscous MEE group (13.6%), in that order. In the purulent MEE group and the mucoid MEE group, the period of observation was significantly shorter in the SAP-positive cases (18.3 ± 20.4 months and 20.2 ± 19.4 months) than in the SAP-negative cases (35.9 ± 24.5 months and 25.4 ± 18.7 months) ($p < .05$). These results suggest that SAP is present in the middle ear cleft and may be a good prognostic predictor of otitis media with effusion in children.

KEY WORDS — immunoassay, middle ear effusion, monoclonal antibodies, otitis media with effusion, surfactant apoprotein.

The pulmonary alveoli are lined with a surface-active material, pulmonary surfactant, that stabilizes the alveoli by lowering surface tension on expiration, thus preventing alveolar collapse. Surface tension–lowering substances have been reported to be present also in the eustachian tube,[1-3] and it was speculated that the substances occurring in the mucosa of the eustachian tube have a definite functional significance, ie, to lower the surface tension and to exert an antiadhesive effect, thus facilitating the normal opening of the tube. In addition, phospholipids, the major surface-active components of pulmonary surfactant, have been also determined in middle ear effusions (MEEs).[4,5] These reports indicate that substances that have a surface tension–lowering activity are present in the eustachian tube washings and that they are mainly composed of phospholipids, which are the major components of pulmonary surfactant. On the contrary, the role of surfactant-like substances in the middle ear cleft in the pathogenesis of otitis media with effusion (OME) has remained unclear. In this study, we measured surfactant apoprotein (SAP) in MEEs by simultaneous immunometric assay with two monoclonal antibodies against human pulmonary SAP and studied the relationship between SAP in MEE and the chronicity of OME.

MATERIALS AND METHODS

Patients. Middle ear effusion samples (100 unilateral and 15 bilateral) were obtained from 115 patients (3 to 14 years old; median, 6 years old) who had undergone routine myringotomy in 130 ears and/or insertion of a ventilation tube under iontophoretic local anesthesia or general inhalation anesthesia for the treatment of OME. The diagnosis of OME was based on the yellow or opaque appearance of the eardrums, the presence of conductive hearing loss, and low compliance of the tympanic membrane with pneumatic otoscopy (B curve or C curve on impedance audiometry). The onset of OME was estimated on the basis of anamnesis as stated by the patients or their parents, or on the basis of documents of referring doctors. The treatment philosophy for patients with OME was as follows.

Patients with OME underwent routine myringotomy and were given proper antibiotics for 7 to 10 days. When the disease did not improve within 3 months in spite of several myringotomies and the medications, the patient was treated with myringotomy and insertion of a ventilation tube. The patients were seen every 2 to 4 weeks at the OME clinic in our department and were examined with a pneumatic otoscope to determine the presence of MEE and the hearing was evaluated. In patients who had no exacerbation of ear infection, the ventilation tubes usually came out within 6 months spontaneously in our series. The tubes in place over 6 months were taken out when the drums showed a normal appearance and then the patients were followed up for another 3 months. Children with normal hearing and no evidence of reaccumulation of effusion for at least 3 months after treatments such as myringotomy and medication, or after the removal of the ventilation tube, were considered to have recovered. We designated this period, ie, from the onset until the complete recovery of OME, as a period of observation. The period of observation

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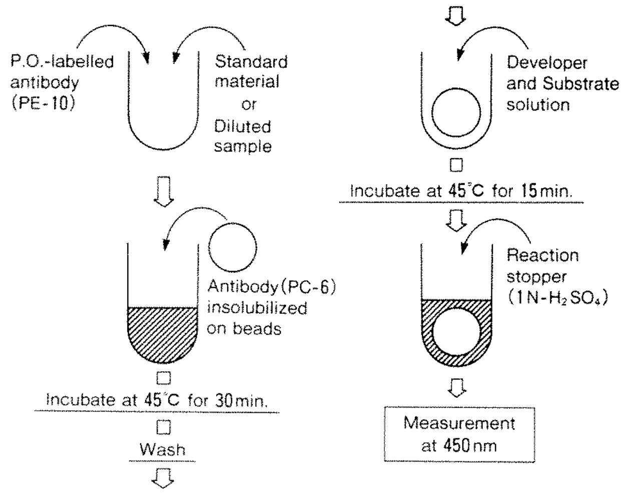
P.O.-labelled antibody (PE-10)
Standard material or Diluted sample
Antibody (PC-6) insolubilized on beads
Incubate at 45°C for 30min.
Wash
Developer and Substrate solution
Incubate at 45°C for 15min.
Reaction stopper (1N-H₂SO₄)
Measurement at 450nm

Fig 1. Two-site simultaneous immunoassay of surfactant apoprotein.

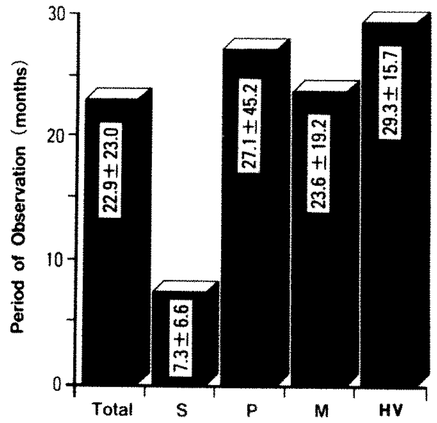

Fig 2. Period of observation for each middle ear effusion. S vs P, $p < .01$; S vs M, $p < .01$; S vs HV, $p < .01$; M vs HV, $p < .05$. S — serous, P — purulent, M — mucoid, HV — hyperviscous.

could include single, chronic, or recurrent episodes or a combination thereof. The incidence of habitual angina, nasal allergy, or chronic sinusitis in patients with OME was assessed by patients' histories, a skin test for allergens, and radiographic findings in the sinuses.

Middle Ear Effusion Samples. The specimens were aspirated under sterile conditions with use of the Juhn Tym-Tap after myringotomy at the first visit and were classified into four types according to the gross appearance: serous (a thin watery fluid), purulent (puslike fluid), mucoid (a thick, viscid, mucuslike fluid), and hyperviscous (very sticky, gluelike fluid). They were then stored at −80°C until use.

Two-Site Simultaneous Immunoassay. Two monoclonal antibodies (PC6 and PE10)[6] against pulmonary SAP were developed and used to measure SAP by means of the two-site simultaneous immunoassay kit (Teijin Institute of Bio-Medicine, Hino, Japan).[7,8] The immunoassay for SAP is shown in Fig 1. Initially, MEE samples were diluted 1:10 with a buffer solution (0.6% sodium dodecyl sulfate, 2% triton X-100, and 0.01 mol/L phosphate-buffered saline, pH 7.2) by vigorously agitating the test tubes on a vortex mixer. Samples that were not mixed homogeneously with the buffer solution including detergents were discarded and were not included in this study. Aliquots (200 μL) of the standard solutions containing 0, 91, 182, and 364 ng/mL of SAP or diluted MEE samples and 200 μL of horseradish peroxidase-labeled monoclonal antibody (PE10) were mixed thoroughly. Plastic beads coated with monoclonal antibody (PC6) were added one by one in each test tube containing the mixture above. After incubation at 45°C for 30 minutes, the reaction mixture was removed by suction with an aspirator and the beads were washed with distilled water four times. Then 400 μL of the mixture of substrate solution (5 mmol/L H_2O_2 and 0.1 mol/L phosphate-citrate buffer, pH 4.0) and developer (0.06% tetramethylbenzidine HCl, pH 2.0) was added to each tube. The tube was incubated at 45°C for 15 minutes and then the reaction was stopped by the addition of 1.0 mL of reaction stopper (1N H_2SO_4). The absorbance of each test tube was measured with an automated double-beam spectrophotometer at 450 nm optical density and copaired with the standard curve. A protein concentration in MEE was also measured with the Protein Assay Kit (Nippon Bio-Rad Laboratory, Tokyo, Japan), and the SAP concentration in MEE was expressed as nanograms per milligram of protein.

Statistical Methods. All contrasts among the various groups were assessed by the Mann-Whitney U test and χ^2 statistics with Yates' correction by using a statistical analysis package for the IBM personal computer (StatPac Gold, Walonick Associates). All conclusions were based on a significance level of $p < .05$.

RESULTS

Gross Appearance of Effusions Versus Clinical Course. Gross examinations of 130 MEE samples yielded the following scores: serous, 16 (12.3%); purulent, 14 (10.8%); mucoid, 78 (60%); and hyperviscous, 22 (16.9%). The period of observation for each group is shown in Fig 2. The mean period of observation for all patients was 22.9 ± 23.0 months. The serous MEE group showed the shortest period of observation (7.3 ± 6.6 months) of the four groups. There was a significant difference in the period of observation between the mucoid MEE group (23.6 ± 19.2 months) and the hyperviscous MEE group (29.3 ± 15.7 months) ($p < .05$). The relationship between the gross appearance of MEE and the incidence of habitual angina, nasal allergy, and chronic sinusitis in OME patients was also investigated (Fig 3). The incidence of habitual angina or nasal allergy in OME patients was relatively low, ie, 5.4% and 15.4%, respectively. On the other hand, the in-

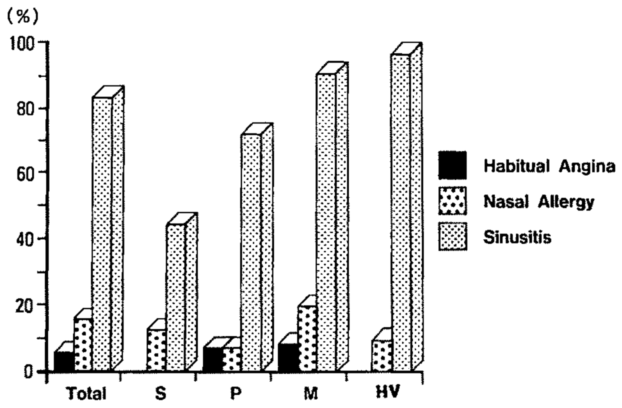

Fig 3. Incidence of habitual angina, nasal allergy, and chronic sinusitis in otitis media with effusion. For abbreviations, see Fig 2.

cidence of chronic sinusitis was quite high (83.1%), especially in the mucoid MEE group (89.7%) and in the hyperviscous MEE group (95.5%).

Surfactant Apoprotein. Figure 4 shows a calibration curve of SAP by the two-site simultaneous immunoassay. The calibration curve was almost superimposable with that of MEE obtained from a patient with OME. The assay was capable of measuring 20 to 400 ng of SAP per milliliter. Surfactant apoprotein was detected in 54 MEE samples (41.5%) and in the remainder the SAP concentration was below the sensitivity of the immunoassay (58.5%). The addition of human serum (2%) to the MEE samples did not change the concentrations or detection rates of SAP. The SAP concentration in positive samples ranged from 300 to 2,460 ng/mL or 5.95 to 709.5 ng/mg of protein (58.9±48.7 ng/mg of protein). To evaluate the masking effect of mucus against SAP, mixtures of SAP-positive serous MEE and SAP-negative hyperviscous MEE were assayed for the SAP level. The results were as follows. Serous MEE samples (SAP levels, 630 ng/mL, 770 ng/mL, and 1,030 ng/mL) mixed with hyperviscous MEE samples (SAP not detected) went to 296 ng/mL, 410 ng/mL, and 580 ng/mL in SAP level, respectively.

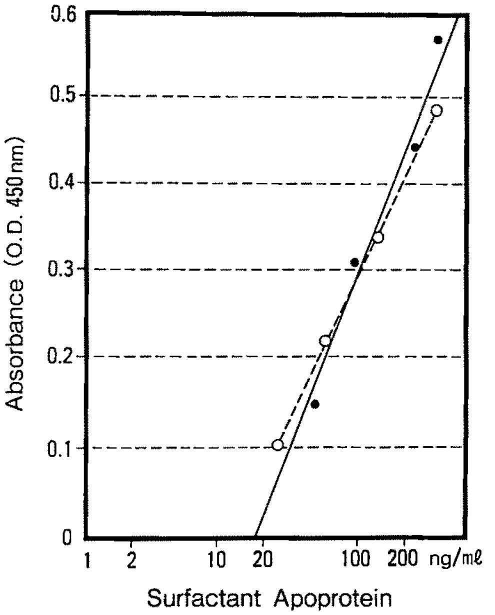

Fig 4. Comparison of calibration curves obtained for dilutions of middle ear effusion (open circles) and purified surfactant apoprotein (closed circles). Values are mean of duplicate assays. O.D. — optical density.

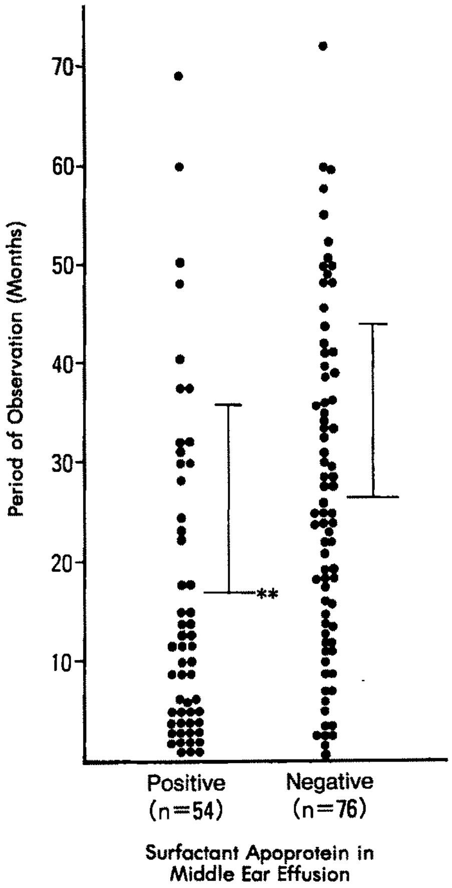

Fig 5. Period of observation versus surfactant apoprotein. ** — p<.01.

Surfactant Apoprotein Versus Clinical Course. There was a significant difference in the period of observation between the SAP-positive cases (17.3± 16.8 months) and the SAP-negative cases (26.2± 22.5 months) (p<.01) (Fig 5). In the SAP-positive cases, however, there was no significant relationship between the SAP concentration in MEE and the clinical course. The percentage of SAP-positive cases in each MEE group is shown in Fig 6. In the serous MEE group 13 of 16 samples (81.2%) were positive for SAP, whereas only 3 of 22 samples (13.6%) in the hyperviscous MEE group were positive for SAP. The relationship between positivity for SAP and the period of observation in each MEE group is shown in Fig 7. In the purulent MEE group and the mucoid MEE group, the period of observation was significantly shorter in the SAP-positive

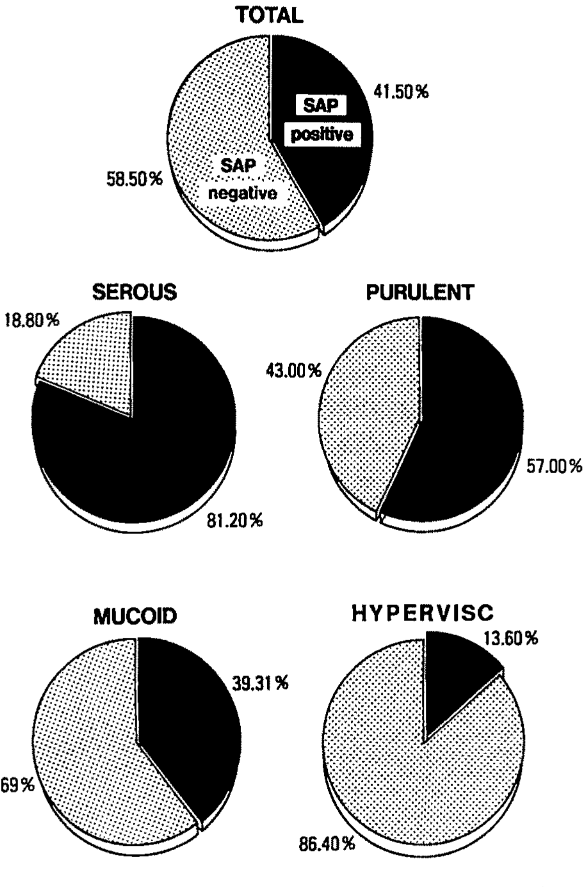

Fig 6. Surfactant apoprotein (SAP) in each middle ear effusion group. HYPERVISC — hyperviscous.

cases (18.3±20.4 months and 20.2±19.4 months) than in the SAP-negative cases (35.9±24.5 months and 25.4±18.7 months) ($p<.05$). The relationship between the incidence of chronic sinusitis and the positivity of SAP is shown in Fig 8. In all MEE groups except the mucoid MEE group, the incidence of chronic sinusitis was significantly higher in the SAP-negative cases than in the SAP-positive cases.

DISCUSSION

It is known that pulmonary surfactant occurs as phospholipid-protein complex in lung lavage and in amniotic fluid, and several attempts to determine the pulmonary surfactant levels by immunologic methods have been reported.[9,10] Kuroki et al[6] have developed monoclonal antibodies against pulmonary surfactant–associated 35-kd protein and 62-kd protein and have established a two-site simultaneous immunoassay by using these monoclonal antibodies.[7,8] The monoclonal antibodies (PC6 and PE10) react only with pulmonary SAP in human amniotic fluid, as well as in human lung lavages, and have not reacted with any other fluids in human organs, including serum, so far.[7,8] Moreover, King et al[11] reported that the change in the concentration of pulmonary SAP in amniotic fluid was approximately parallel with that of the phospholipid concentration. These reports indicate that the

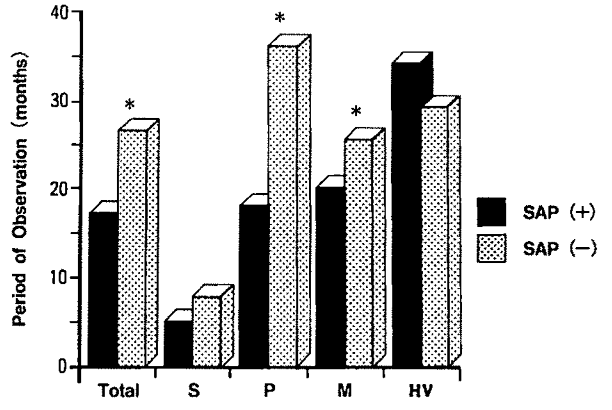

Fig 7. Period of observation and presence of surfactant apoprotein (SAP) in each middle ear effusion group. * — $p<.05$. For abbreviations, see Fig 2.

monoclonal antibodies against SAP will be very useful for investigations of human pulmonary surfactant. We applied this immunoassay with the monoclonal antibodies to investigate the presence of SAP in MEEs. The result of this study showed that SAP was present in an appreciable number of MEE samples obtained from children with OME. It is important to note that this result does not always mean that in the SAP-positive MEEs surface tension–lowering substances analogous to pulmonary surfactant are present, and does not preclude the possibility that other surface-active substances are also present in the SAP-negative MEEs. Grace et al[5] reported that appreciable quantities of phospholipids were present in all middle ear aspirates studied; phosphatidylcholines formed the predominant component in children. Whether substances in MEEs that react with the monoclonal antibodies are the same as pulmonary SAP is now under investigation; however, the SAP in MEEs is likely to occur surrounded with high levels of lipids. In addition, in dealing with MEE samples there might be a sampling problem due to the difficulty of solubilizing substances from mucoid or hyperviscous effusions. To resolve these issues, in the immunoassay of SAP in MEEs, detergents such as sodium dodecyl sulfate and triton X-100 and a relatively high reaction temperature that does not affect the immunoreaction (45°C) were used. Moreover, the SAP level in mixtures of serous, high-SAP samples with hyperviscous, SAP-negative samples was approximately half of the initial (original) level. This result precludes the possibility that low SAP levels in mucoid or hyperviscous MEE samples are related to failure of recovery of SAP by the masking effect of mucus in effusion.

In our study, SAP was found in about 42% of MEEs. It seemed essential to determine whether the SAP was actively produced in the middle ear cleft or was derived from plasma. Our observations revealed that addition of human serum to MEE samples did not change concentrations or detection rates of SAP. This result indicated that human serum did not con-

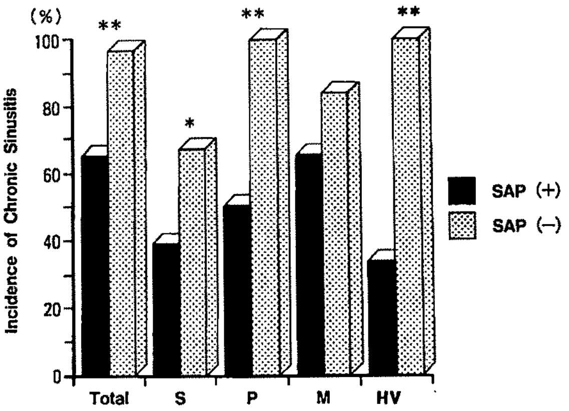

Fig 8. Relationship between presence of surfactant apoprotein (SAP) in middle ear effusion and chronic sinusitis. * — $p<.05$, ** — $p<.01$. For abbreviations, see Fig 2.

tain SAP; hence, this was not the source of SAP in MEEs. On the basis of our findings, it is likely that the SAP may be locally produced, ie, in the middle ear mucosa or in the eustachian tube.

The SAP level in MEEs in our study was considerably lower than that in amniotic fluids (0.84 to 13.1 μg/mL)[7] and was found to have no significant correlation with the clinical course of OME in the SAP-positive cases. On the other hand, the positivity of SAP in MEEs showed a good correlation with the clinical course of OME. In all MEE samples, the period of observation of the SAP-positive cases was significantly shorter than that of the SAP-negative cases.

Senturia[12] originally classified MEEs into five types, ie, serous, seropurulent, mucopurulent, and mucoid, on the basis of history, otologic and physical findings, and cytologic and biochemical data. Because of the difficulty of classifying MEEs into five types only by gross appearance, we classified MEEs into four types, ie, serous, purulent, mucoid, and hyperviscous. The hyperviscous MEEs may be classified as mucoid; however, when hyperviscous MEEs are included in the mucoid group, over two thirds of the MEEs in our study will be classified as mucoid. We separated hyperviscous MEEs from mucoid MEEs because they were much more viscous than mucoid MEEs and sometimes difficult to aspirate thoroughly from the middle ear cavity. In the comparison of period of observation by type of MEE, the serous MEE group showed the shortest period of the four groups, whereas no significant differences were found among the other three groups. The percentage of positivity for SAP was the highest in the serous MEE group and the lowest in the hyperviscous MEE group. These data suggest that SAP is inversely correlated with the mucus content of MEEs. This could be because low SAP levels are associated with more severe disease, which in turn leads to increased mucus production.

Generally, in children, chronic inflammations of the ear, nose, and throat tend to complicate each other. In our study the incidence of chronic sinusitis was very high in children with OME, possibly because the majority of the patients were remitted to us from general practitioners as having resistant cases complicating chronic sinusitis. It was notable that the incidence of chronic sinusitis complicated with OME was significantly lower in the SAP-positive cases than in the SAP-negative cases in all MEE groups except the serous group. These results suggest that chronic infections in the upper respiratory airways such as chronic sinusitis form the bacterial foci in the nasopharyngeal space causing tubotympanitis, thus reducing the production of SAP in the middle ear mucosa or in the eustachian tube.

White[13] reported the effect of exogenous surfactant on eustachian tube function in the rat and suggested a physiologic role for surface tension–lowering substances in tubal function. In addition, apoprotein in the surfactant is thought to be of importance to the surface tension–lowering action,[14] and an analysis of surfactant from the eustachian tube of guinea pigs also reveals a portion of proteins.[15] In consideration of these facts, our data suggest that the SAP level may reflect the surfactant activity in the human eustachian tube to some degree, and that low SAP levels may be associated with impaired tubal function, which in turn leads to more severe disease.

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EXTRACRANIAL OPTIC NERVE DECOMPRESSION MEETING

An Extracranial Optic Nerve Decompression Meeting will be held Nov 2-3, 1991, in Boston. For further information, contact Michael P. Joseph, MD, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114; (617) 573-3192.

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INTERNATIONAL HEARING AID CONFERENCE
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An International Hearing Aid Conference will be held June 15-17, 1993, at The University of Iowa, Iowa City, Iowa. For information on registration and accommodation, contact the Conference Center, University of Iowa, Memorial Union, Iowa City, IA 52242; (319) 335-3231, Fax (319) 335-3407. For information on Call for Papers, contact Regina Tisor (319) 356-2471, Fax (319) 356-4547. We have applied for Continuing Education Units from ASHA and HAIC.

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CEREBRAL AUTOREGULATION IN PATIENTS WITH ORTHOSTATIC HYPOTENSION

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The present study was conducted to determine the relationship between autonomic nervous function and cerebral autoregulation in 17 patients with vertigo or dizziness. Autonomic nervous function was examined by measuring systolic blood pressure response and dynamic electrocardiogram R-peak to R-peak response upon standing up. Regional cerebral autoregulation was examined by comparing cerebral blood flow in both supine and standing positions with single photon emission computed tomography. Moreover, the relationship of each of these three measures to postural provocation of symptoms was studied. The findings indicated that autonomic nervous dysfunction does not influence cerebral autoregulation, but autonomic nervous dysfunction was related to postural provocation of symptoms. This might indicate that impaired autoregulation in the brain stem or the peripheral vestibule, which could not be detected by the single photon emission computed tomography, played a role in inducing vertigo or dizziness in patients with orthostatic hypotension.

KEY WORDS — autonomic nervous system, autoregulation, dizziness, orthostatic hypotension, single photon emission computed tomography, vertigo.

INTRODUCTION

Between 1984 and 1986, a total of 1,291 patients underwent otoneurologic examinations at our hospital because of complaints of vertigo, dizziness, and/or dysequilibrium. Of these patients, 131 showed systolic orthostatic hypotension (SOH). Presently, there are two different views on cerebral autoregulation and autonomic nervous dysfunction. Caronna and Plum[1] and Meyer et al[2] found that patients with pure autonomic failure or multiple system atrophy were more likely to show impaired cerebral autoregulation, while others[3-5] noted that patients with pure autonomic failure and multiple system atrophy show preserved cerebral autoregulation. In this study, 17 patients who complained of vertigo or dizziness were examined to clarify the relationship between the autonomic nervous system and cerebral autoregulation.

METHODS

Systolic Blood Pressure Response Upon Standing. Systolic blood pressure upon changing from a supine to a standing position was measured by the Schellong test maneuver.[6] The patient was instructed to remain in a supine position for at least 10 minutes and to then actively stand up and remain standing for 10 minutes. Systolic blood pressure was measured just before standing up, just after standing up, 5 minutes after standing up, and 10 minutes after standing up. A patient was considered to have SOH if at least one of the standing systolic blood pressure readings was more than 21 mm Hg lower than that just before standing.[6-9]

Dynamic Electrocardiogram R-Peak to R-Peak Response. The electrocardiogram R-peak to R-peak (ECG RR) intervals shorten quickly upon active standing up in healthy volunteers. Dynamic change in ECG RR interval is determined by subtracting the ECG RR interval just after standing up from that recorded just before standing up. The normal range of dynamic ECG RR response varies with age,[10] as autonomic nervous system function has been found to undergo age-related changes.[11]

Regional Cerebral Autoregulation. Regional cerebral autoregulation was measured by comparing regional cerebral blood flow while in the supine and standing positions. Regional cerebral blood flow measurement, using N-isopropyl-p-[^{123}I]iodoamphetamine (IMP) and single photon emission computed tomography (SPECT), was performed once and repeated 7 days later. In the first measurement, IMP was injected intravenously, and the patient was in a supine position for at least 20 minutes. After 7 days, washout was complete, and the second measurement was performed. In the second measurement, the patient was requested to actively stand up after having been in a supine position, at which time IMP was injected intravenously. In both measurements, the position, ie, supine for the first measurement and standing for the second, was maintained for 20 minutes after IMP injection, at

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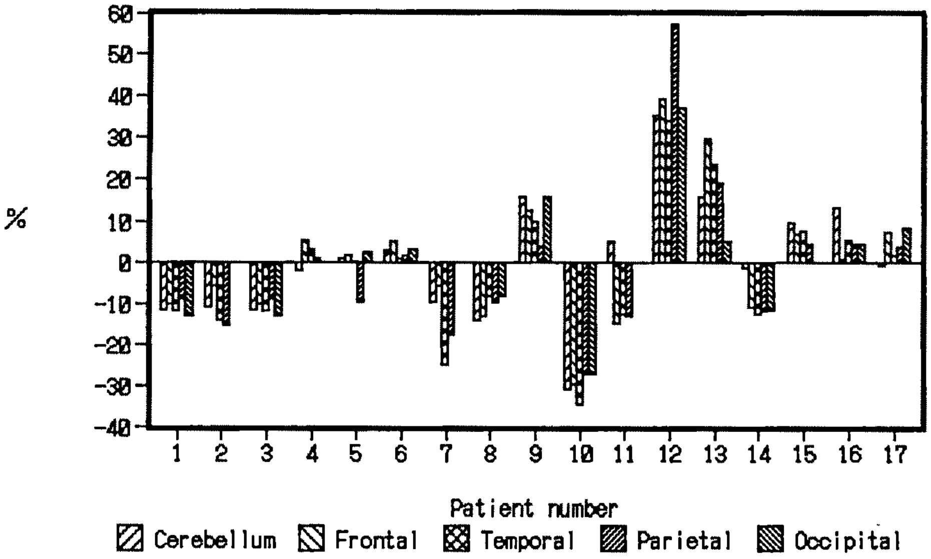

Change in regional cerebral blood flow. Values on ordinate indicate percent change in cerebral blood flow upon changing from supine to standing position. Percent change less than −10 was considered to indicate regional cerebral dysautoregulation.

which time the IMP had been almost completely absorbed by the brain. Five cerebral regions, ie, the cerebellum and frontal, temporal, parietal, and occipital lobes, were determined on the basis of analysis of axial tomograms on an image-processing display. In each region, a square with an area of 10 x 10 mm was chosen bilaterally. Radioactivity was counted in bilateral squares in the same region. Sometimes a region had to be excluded from analysis because of poor imaging. The relative change in cerebral blood flow (100.0 x [radioactivity counts in a region in the standing position − radioactivity counts in a region in the supine position]/[radioactivity counts in a region in the supine position]) was calculated. A change of less than −10% (see Figure) was considered to indicate pathologic regional cerebral autoregulation, ie, regional cerebral dysautoregulation (RCDA).[12]

TABLE 1. PATIENT PROFILES

			Blood Pressure				
Patient	*Age*	*Sex*	*A*	*B*	*C*	*D*	*Main Disease or Complaint*
1	77	F	110	80	30	+	Hypertension
2	66	M	124	89	35	+	SCD
3	66	M	117	83	34	+	Hemiplegia
4	70	F	137	91	46	+	VBI, cerebral arteriosclerosis
5	72	F	125	102	23	+	None
6	40	M	141	120	21	+	None
7	70	M	225	154	71	+	Cerebellar infarction
8	74	M	140	113	27	+	None
9	54	M	155	130	25	+	None
10	71	M	142	118	24	+	Brain stem infarction
11	67	M	148	118	30	+	Myocardial hypertrophy
12	38	F	120	50	70	+	Headache
13	50	F	108	87	21	+	VBI
14	53	M	131	115	16	−	None
15	37	F	111	97	14	−	Traumatic brain stem injury
16	26	M	119	115	4	−	SCD
17	44	M	121	109	12	−	None

A — systolic blood pressure just before standing, B — lowest systolic blood pressure while standing, C — A minus B, D — systolic orthostatic hypotension status (positive or negative), SCD — spinocerebellar degeneration, VBI — vertebrobasilar insufficiency.

PATIENTS

Seventeen patients without well-defined peripheral vestibular disorders (for example, Meniere's disease) were studied. All patients underwent otoneurologic examinations, including blood pressure measurement by the Schellong test maneuver. Patients (11 men and 6 women) ranged in age from 26 to 77 years. Thirteen patients (patients 1 to 13) showed SOH, and the 4 controls (patients 14 to 17) did not show SOH. Patient profiles, including main disease or complaint, are listed in Table 1.

RESULTS

Table 2 shows symptoms (vertigo or dizziness) and postural provocation status (PPS). Seven patients complained of vertigo and 10 of dizziness. Symptoms of 11 patients were provoked by postural change (PPS+), and those of 5 were not (PPS−). Table 3 shows the results of dynamic ECG RR re-

TABLE 2. SYMPTOMS OF PATIENTS AND POSTURAL PROVOCATION STATUS

Patient	*Symptom*	*Provoked by Postural Change**
1	Dizziness	+
2	Vertigo	+
3	Dizziness	+
4	Vertigo	+
5	Vertigo	+
6	Dizziness	+
7	Dizziness	?
8	Vertigo	−
9	Dizziness	+
10	Vertigo	+
11	Dizziness	+
12	Vertigo	+
13	Dizziness	+
14	Vertigo	−
15	Dizziness	−
16	Dizziness	−
17	Dizziness	−

*From supine to standing position.

TABLE 3. DYNAMIC ECG RR RESULTS

	Dynamic ECG RR Response		
Patient	*NV*	*NR*	*Classification**
1	5	7-13	+
2	3	8-12	+
3	7	8-12	+
4	2	7-13	+
5	6	7-13	+
6	12	9-15	−
7	NP		
8	11	7-13	−
9	6	8-13	+
10	7	7-13	−
11	7	8-12	+
12	7	9-20	+
13	17	8-13	+
14	9	8-13	−
15	18	9-20	−
16	9	10-16	+
17	18	9-15	+

ECG RR — electrocardiogram R-peak to R-peak, NV — numeric value, NR — normal range (mean±SD), NP — not performed because of arrhythmia.

*Plus indicates abnormal ECG RR response.

TABLE 4. CHI-SQUARE TESTS BETWEEN SOH AND RCDA AND BETWEEN ECG RR AND RCDA

	RCDA +		*RCDA −*
SOH +	7		6
SOH −	1		3
p		NS	
ECG RR +	4		7
ECG RR −	3		2
p		NS	

RCDA — regional cerebral dysautoregulation, SOH — systolic orthostatic hypotension, ECG RR — electrocardiogram R-peak to R-peak.

sponse. Numeric values of the dynamic ECG RR response ranged from 2 to 18. Classification of dynamic ECG RR response as normal (−) or abnormal (+) was based on normal ranges found in 51 healthy volunteers ranging in age from 20 to 80 years.[10] The dynamic ECG RR response was not examined in patient 7 because of the presence of arrhythmia. Eleven patients showed an abnormal dynamic ECG RR response. The Figure shows RCDA. In 8 of 17 patients (patients 1, 2, 3, 7, 8, 10, 11, and 14), RCDA was observed in one or more regions. The remaining 9 patients did not show RCDA in any region. Table 4 shows that there was no significance in correlations either between SOH and RCDA or between ECG RR and RCDA. Table 5 shows that there was not a significant correlation between RCDA and PPS, but there was a significant correlation ($p<.001$) between SOH and PPS and a high correlation ($p = .0944$) between ECG RR and PPS.

DISCUSSION

Shy-Drager syndrome, which is characterized by dizziness on standing, gait disturbance, and sexual impotence, is a representative disease manifesting SOH.[2,13] Besides this syndrome, SOH caused by autonomic nervous dysfunction is occasionally encountered in patients with vertigo, dizziness, and/or dysequilibrium.[14] The ECG RR responses are diminished in patients with autonomic neuropathy, as in those with diabetes mellitus.[11,15] Therefore, measurement of static and dynamic ECG RR responses is also assumed to indicate the functioning of the autonomic nervous system. The relationship between the autonomic nervous system and cerebral autoregulation has been controversial.

Cerebral autoregulation acts to maintain constant blood flow despite changes in perfusion pressure.[16] Intracranial blood vessels have been shown to be capable of dilating to maintain cerebral blood flow when extracranial vasospasm occurs secondary to subarachnoid hemorrhage or acute hypotension.[17] Caronna and Plum[1] reported that subjects with pure autonomic failure show destruction of postganglionic neurons in the autonomic nervous system and impaired cerebral autoregulation. Meyer et al[2] also found impaired cerebral autoregulation in patients with multiple system atrophy. Contrary to these reports, Brooks et al,[3] Nanda et al,[4] and Thomas and Bannister[5] noted that pure autonomic failure and multiple system atrophy subjects show preserved cerebral autoregulation; these findings can be accounted for by the fact that the arterioles of the cerebral blood vessels are poorly innervated by the autonomic nervous system. The present result (Table 4) is in accordance with reports concluding that autonomic nervous dysfunction does not influence cerebral autoregulation. This conclusion also implies that cerebral autoregulation is not controlled by the autonomic nervous system. However, dysautoregulation can occur in both the central nervous system[2] and the brain stem[18] or peripheral vestibule[19] as well. The organ most often affected in patients with vertigo or dizziness is the brain stem or peripheral vestibule. In the present study, postural provocation of the symptoms of vertigo and dizziness was found not to be dependent on RCDA, but was found to be significantly or highly correlated with autonomic nervous dysfunction (Table 5). These findings might suggest that dysautoregulation of the brain stem or peripheral vestibule could occur in patients with autonomic nervous dysfunction, in which case, because of the small size of the brain stem and peripheral vestibule, SPECT is not useful in detecting the source of dys-

TABLE 5. CHI-SQUARE TEST BETWEEN RESULTS OF THREE EXAMINATIONS AND PPS

	PPS +		*PPS −*
RCDA +	5		2
RCDA −	6		3
p		NS	
SOH +	11		1
SOH −	0		4
p		<.001	
ECG RR +	9		2
ECG RR −	2		3
p		.0944	

PPS — postural provocation status. Other abbreviations as in Table 4.

autoregulation.

Radiolabeled amines, such as IMP, cross the blood-brain barrier by inhibiting serotonin and norepinephrine in synaptosomes.[20,21] Kuhl et al[22] showed nearly complete IMP uptake on the first passage through the brain of a monkey following intracarotid injection. Winchell et al[16] proposed that the analysis of the initial distribution of IMP in humans may be useful for assessing relative regional brain perfusion on the basis of the findings that extraction efficiency in the rat brain is high, washout is slow, and brain-blood ratio is high. Consequently, IMP is the most commonly used radioisotope for measuring regional cerebral blood flow. However, free diffusion through the blood-brain barrier without an active transport mechanism and slow diffusion back into the blood have so far not been confirmed, absolute quantification of SPECT findings remains very problematic,[22] and the most practical compromise at present is thus to conduct semiquantitative analyses.[23] Also, the present study discloses that SPECT may not be suitable for measuring blood flow in the brain stem or peripheral vestibule, because its resolution capacity is not sufficient. A technique for measuring blood flow in a small organ (the brain stem or the peripheral vestibule) needs to be developed.

In conclusion, cerebral dysautoregulation was not influenced by the autonomic nervous dysfunction, but it might be possible that dysautoregulation in the brain stem or peripheral vestibule caused vertigo or dizziness in the patients with SOH.

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ROSAI-DORFMAN DISEASE INVOLVING THE PREMAXILLA

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Sinus histiocytosis with massive lymphadenopathy (SHML), introduced in 1969 by Rosai and Dorfman, is a benign and self-limited (albeit often relapsing) pseudolymphomatous entity with characteristic histopathologic features. Although the presence of massive cervical lymphadenopathy often aids in the diagnosis of SHML, the disease may present in the absence of detectable nodal involvement (Rosai-Dorfman disease). Therefore, the physician must be aware of such extranodal presentations. A recent review of the 365 cases in the SHML registry has revealed a preponderance of eyelid and/or orbital involvement; only 26 cases were found with subcutaneous or soft tissue involvement in areas other than the eyelid and/or orbit. This is a case report of one such atypical presentation of SHML without clinically apparent lymphadenopathy or eyelid or orbital involvement.

KEY WORDS — lymphadenopathy, Rosai-Dorfman disease, sinus histiocytosis.

INTRODUCTION

Sinus histiocytosis with massive lymphadenopathy (SHML), introduced in 1969 by Rosai and Dorfman,[1] is a distinct benign pseudolymphomatous entity with characteristic histologic features.[2,3] The most common site for lymphadenopathy in SHML is cervical. However, other nodal sites have been implicated in up to 78% of patients.[1,3-6] Mediastinal, axillary, inguinal, and retroperitoneal nodes may be affected.[2] The lymphadenopathy, when present, tends to be painless and bilateral. The rare finding of unilateral upper, deep cervical, or unilateral submandibular lymphadenopathy may pose a difficult diagnostic dilemma in differentiating between SHML and other neck masses.[3,7,8] Perhaps an even more misleading presentation occurs when the disorder presents in the absence of clinically apparent lymphadenopathy.[2] The absence of lymphadenopathy makes the term SHML inappropriate; therefore, the eponym of Rosai-Dorfman disease is preferable.[4,9]

Extranodal tissue involvement occurs in 25% to 40% of cases[4,9,10] and may involve bone, testes, orbit, eyelid, upper respiratory tract, salivary glands, skin, lungs, adnexa, kidney, central nervous system, peri-

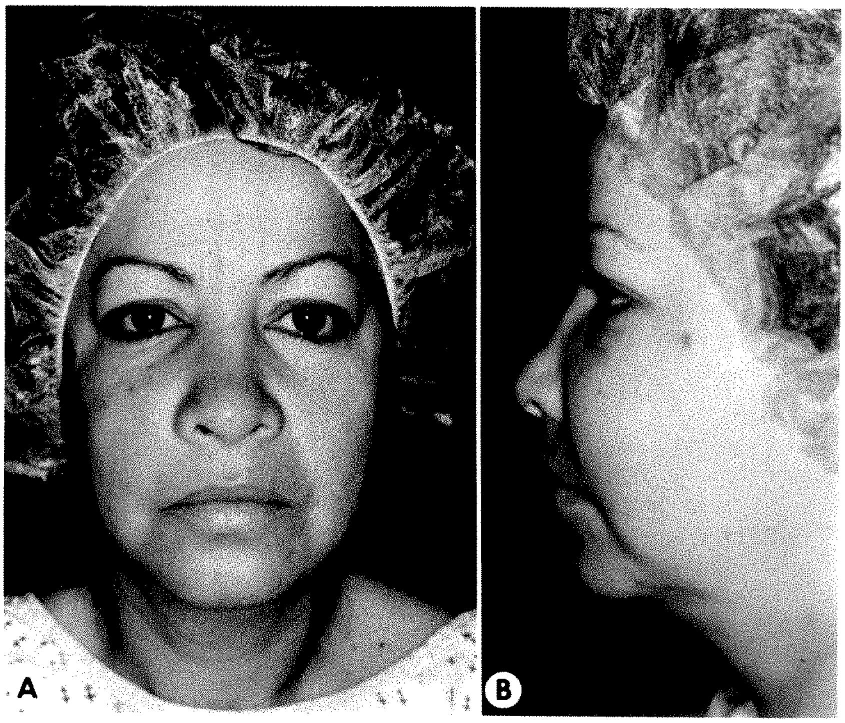

Fig 1. Preoperative views of patient. A) Frontal. B) Lateral.

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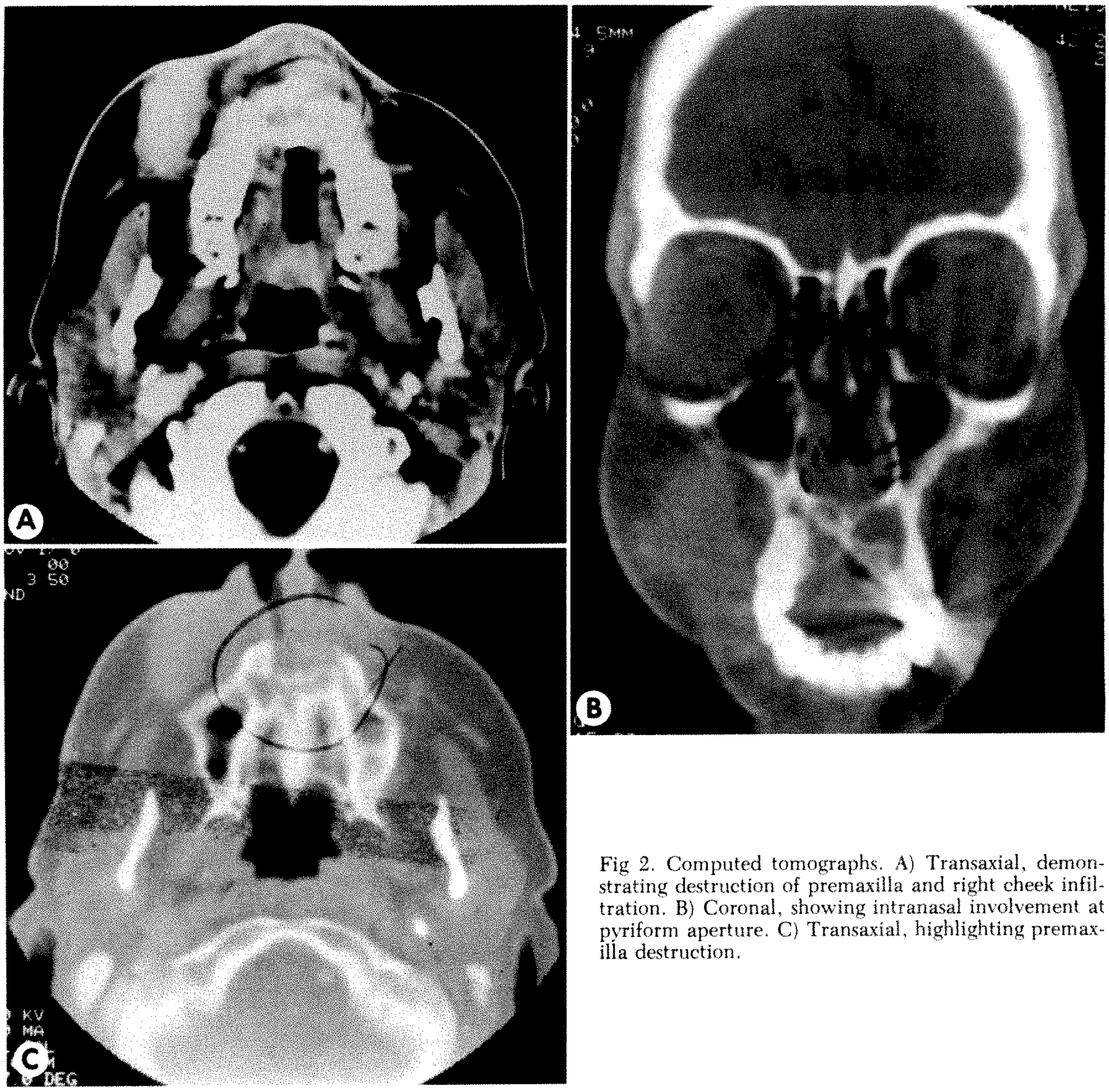

Fig 2. Computed tomographs. A) Transaxial, demonstrating destruction of premaxilla and right cheek infiltration. B) Coronal, showing intranasal involvement at pyriform aperture. C) Transaxial, highlighting premaxilla destruction.

toneum, thyroid, small intestine, and joints.[2-6,11-16] Multisystemic involvement usually occurs but does not signify a less favorable prognosis.[4,6] However, extranodal involvement appears to be more common in patients with immunologic abnormalities, which may themselves contribute to a poorer prognosis.[7]

Clinical signs and symptoms of SHML include fever, weight loss, tonsillitis, rhinorrhea, nasal discharge and obstruction, otitis media, cellulitis, and mild to moderate hepatosplenomegaly.[1,3-7,10,12] Laboratory findings in SHML cases include anemia, an elevated erythrocyte sedimentation rate, leukocytosis with a neutrophilia, and polyclonal hypergammaglobulinemia.[2,10] The histologic findings of sinusoidal histiocytosis with lymphophagocytosis and plasma cell proliferation (seen within biopsies of lymph nodes and/or extranodal sites), along with the immunocytochemical demonstration of S-100 protein reactivity within these histiocytes, aids in the diagnosis.[2,9,17-19] Strong responses to S-100 protein cytoplasmic and nuclear staining, weak reactivity to α1-antichymotrypsin, and no reactivity with Leu, leukocyte common antigen, M-1, and muramidase stains have been described.[2,7] Large, atypical histiocytes at times may resemble Reed-Sternberg cells, further exacerbating diagnostic confusion.[3,7]

Epidemiologic evaluation of SHML shows a slightly less than 2:1 male-to-female ratio. Also, a predominance of cases involve children and adolescents: 62% of presentations occurred in patients under 10 years of age, and 81% of presentations occurred in patients under 20 years of age. However, an age range as wide as 1 to 69 years of age has been noted.[4-8,10,12,20] Finally, no one socioeconomic class appears to predominate,[9] and although it was originally thought to be more prevalent among blacks, current reports note that SHML affects white and black persons in approximately equal numbers.[7,9]

Despite various theories, the cause of SHML remains unknown. Early findings of elevated Epstein-

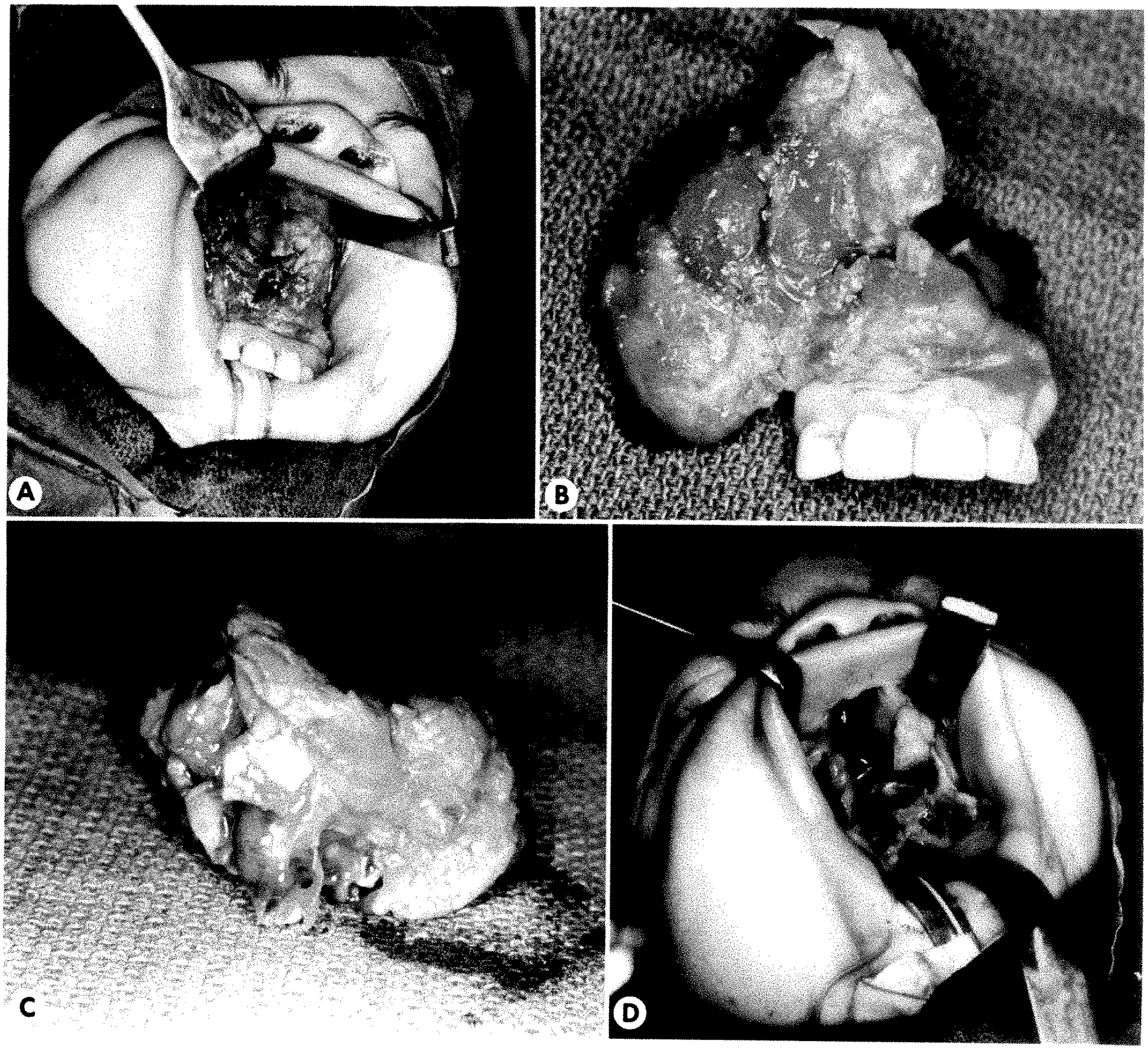

Fig 3. Operation. A) Midfacial degloving approach to nose, premaxilla, and soft tissues of cheek. B) Anterior view of surgical specimen including premaxilla, floor of nose, right anterior maxilla with overlying soft tissues, and right nasal bone. C) Posterosuperior view of surgical specimen, showing antrum and associated soft tissues. D) Surgical defect.

Barr virus and measles titers are viewed as resulting from an abnormal cell-mediated response.[10] High antibody titer to the *Klebsiella* antigen has also been noted.[10,12,21] A direct Coombs reaction is seen in the majority of patients who have immunologic changes.[10,20] Buchanan et al,[22] as well as Bankaci et al,[23] postulate a possible genetic predisposition to SHML through their observation of the occurrence of SHML in pairs of siblings.

Although appropriate therapy is debatable, local surgery seems to be the most promising treatment modality.[10] Local surgery also appears to be the most effective treatment for SHML-induced bone lesions.[21] Antibiotics, antituberculosis medications, various chemotherapeutics, irradiation, and steroids have all apparently failed as consistently effective treatment and therapeutic options.[9,10,21]

As discussed above, the diagnosis of SHML (or Rosai-Dorfman disease in the absence of lymphadenopathy) is difficult to make without the characteristic nodal involvement. The soft tissue involvement of SHML or Rosai-Dorfman disease was previously thought to be limited almost entirely to the eyelid and orbit.[15] However, a review of the SHML registry (365 cases) yields 26 cases with soft tissue involvement other than that of the eyelid or orbit.[9] In this paper we report an unusual case of Rosai-Dorfman disease involving the soft tissues and bones of the face other than the orbit and eyelid, and without clinically apparent cervical or other nodal lymphadenopathy.

CASE REPORT

A 42-year-old woman presented with the chief

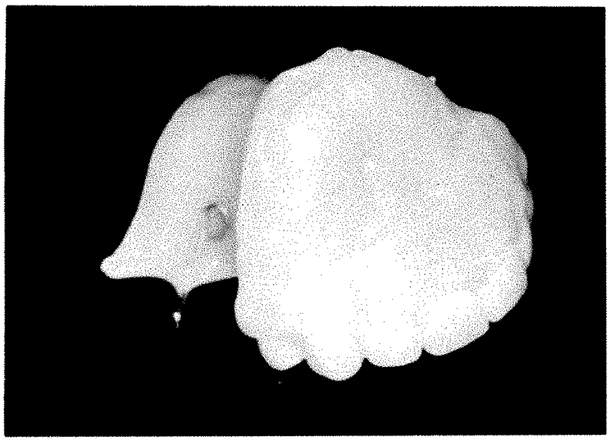

Fig 4. Prosthesis used.

complaint of a right nasofacial mass in June 1988. Two years before, she had undergone an incisional biopsy of a right nasolabial cyst. When the mass regrew 15 months later, she underwent a Caldwell-Luc procedure in Colombia. The mass again recurred but within a shorter time frame and was associated with pressure in the right orbit and right side of the face as well as right nasal obstruction. Her medical history included diet-controlled diabetes. Head and neck examination disclosed marked induration and thickening in the subcutaneous tissues overlying the right maxillary sinus, extending along the right nasofacial groove toward the medial canthal area. An underlying mass appeared to bulge and distort the gingivobuccal sulcus (Fig 1). A computed tomographic scan demonstrated a markedly enhancing mass in the right cheek, anterior to the maxilla (Fig 2A). This mass extended medially to involve the soft tissues of the right anterior naris and over the right nasal bone (Fig 2B). There was fragmentation of the premaxilla with enhancing soft tissue density within the region of bone loss, and this process continued through the hard palate into the nasal floor. The anterior portion of the bony nasal septum was also seen to be destroyed by the mass (Fig 2C).

On July 13, 1988, the patient was taken to the operating room and the lesion was approached through a degloving approach (Fig 3A). The involved cheek, soft tissues, premaxilla, palate, right maxilla, right nasal bone, and nasal floor were all resected (Fig 3B,C). A prefabricated palatal prosthesis was wired into position to occlude the defect (Fig 3D) and achieve oral competence. This was later replaced with a final prosthesis (Fig 4).

The specimen was reviewed by several pathologists, who concurred with a diagnosis of a nonspecific, chronic inflammatory lesion with a florid plasmocytic infiltrate in the background of fibrous histiocytic proliferation. The alternation of clusters of mature lymphocytes and plasma cells with expanses of large histiocytes with abundant clear cytoplasm containing phagocytosed lymphocytes was typical of Rosai-Dorfman disease. There was evidence of erosion and ulceration with destruction of chondroid and osseous tissues (Fig 5). Further microscopic examination revealed infiltrates in respiratory mucosa, striated muscle, subcutis, and the nasal and maxillary bones. These infiltrates showed variability in cellularity and in the amount of collagenized stroma. Immunostaining revealed positive reactivity against α1-antichymotrypsin and S-100 protein in the histiocytic cells. Special stains (Brown & Hopps, Grocott, Steiner, acid-fast bacillus, Giemsa, and periodic acid–Schiff) failed to reveal any microorganisms.

In January 1989, two lesions recurred in the soft tissues of the cheek. These were locally resected. The patient then underwent radiotherapy to the middle of the face (40 Gy in 20 fractions over 30 days through a 3 x 12-cm anteroposterior field by means of the 5-MeV Orion linear accelerator). She now remains disease-free. Photographs of her current appearance are presented (Fig 6).

COMMENT

The lack of lymphadenopathy in our case warrants the terminology of Rosai-Dorfman disease rather than SHML. The case is an unusual presentation of soft tissue involvement exclusive of the orbit and eyelid. In previously reported cases of SHML and/or Rosai-Dorfman disease, the most common head and neck manifestations include the nasal cavity, salivary glands, tongue, pharynx, paranasal sinuses, and tonsils.[7] The majority of these patients manifested two or more head and neck structure sites. The most common complaints from such patients include nasal obstruction, epistaxis, dyspnea, and salivary gland enlargement.[7] Our patient had multiple head and neck site manifestations, including involvement of the nose, paranasal sinuses, and minor salivary glands. The osseous involvement in our case included bony destruction of the premaxilla, anterior portion of the right hard palate, and anterior bony septum. Prior reports of such osseous manifestations seem to suggest the possibility of secondary osseous invasion by contiguous soft tissue disease.[4,6] Our perioperative findings are consistent with this mode of pathophysiology.

DISCUSSION

Sinus histiocytosis with massive lymphadenopathy is regarded as a benign, pseudolymphomatous disorder of unknown cause and has been thought by some to represent the result of an abnormal immune reaction.[2,20,24,25] Clinically, the disease is characterized by an often protracted although benign course.[1-3] Spontaneous remissions may occur; however, relapses have also been noted.[9] Recent reports document that infiltrates of SHML can cause death.[2,9] Foucar et al[9] attribute fatalities associated with SHML to related immunologic disorders.

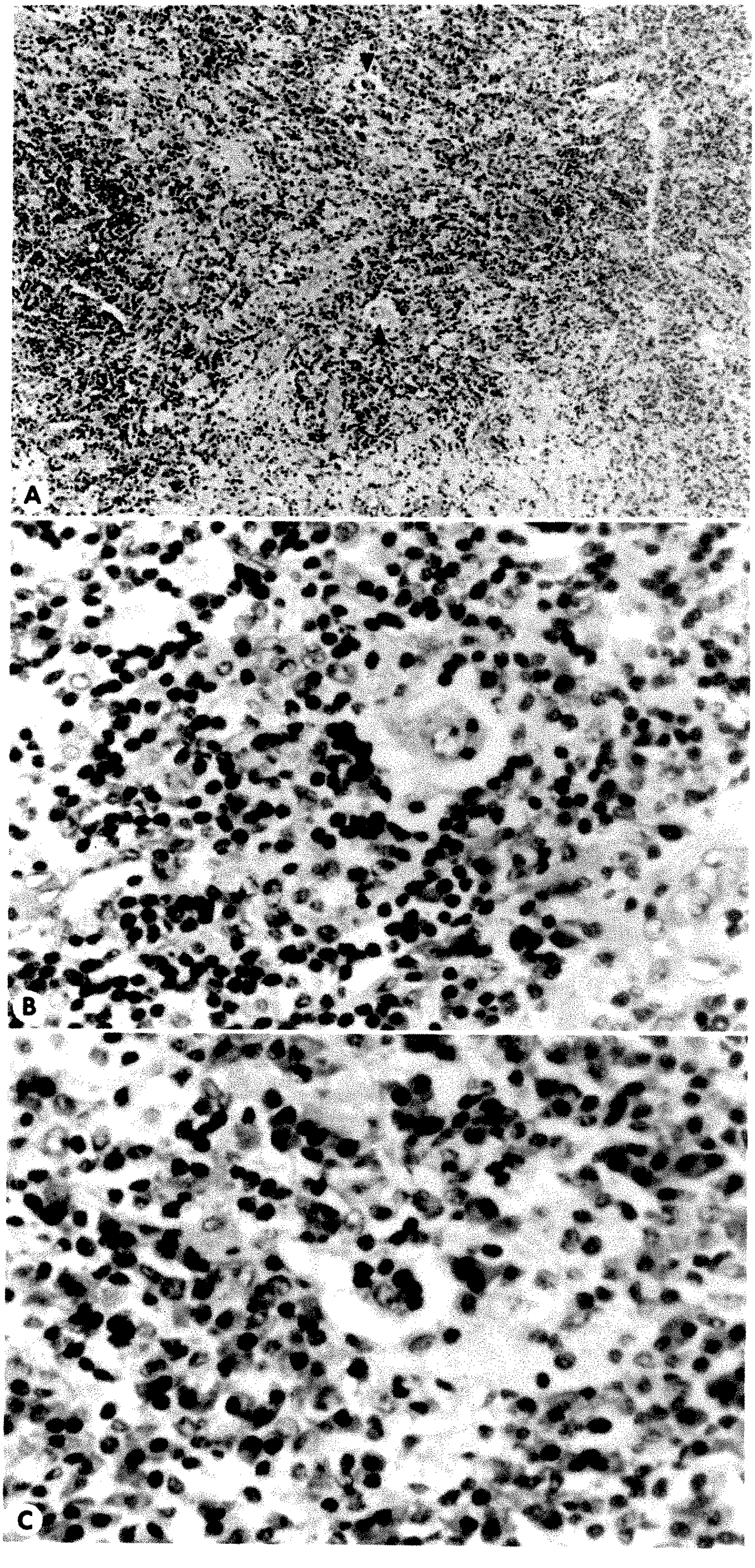

Fig 5. Histologic specimens. A) Premaxillary soft tissue infiltrate from cellular area showing polymorphous cell population that includes many histiocytes, two of which are highlighted with arrowheads (H & E, original x160). B) High-power view showing histiocyte with several phagocytosed lymphocytes (H & E, original x400). C) Additional histiocyte under high magnification (H & E, original x400).

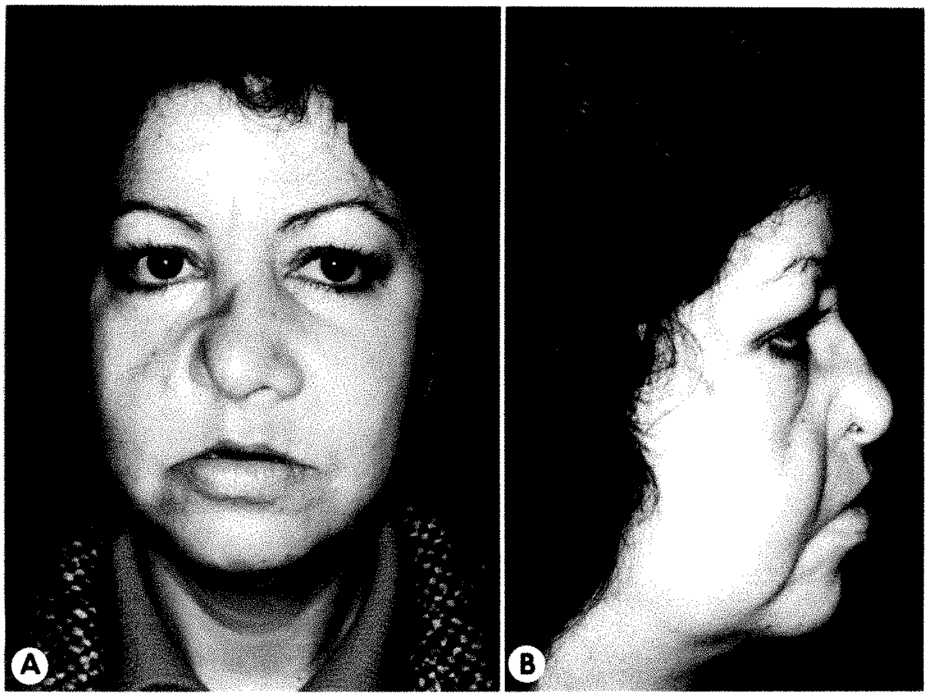

Fig 6. Final appearance of patient. A) Frontal view, without prosthesis. B) Lateral view of affected side, with patient wearing prosthesis.

A review of the SHML registry has yielded 26 patients with soft tissue involvement of the head and neck other than the eyelid or orbit.[9] These patients with SHML and soft tissue disease are significantly older at the time of onset (mean age, approximately 40 years) than the remainder of the registry patients (mean age, 19.7 years).[2,9] A number of patients with soft tissue involvement also have associated lymph node disease diagnostic of SHML. These findings strengthen the suggestion that such soft tissue abnormalities, often difficult to diagnose, are in fact part of the same disease process as isolated soft tissue SHML or Rosai-Dorfman disease.[9]

Sinus histiocytosis with massive lymphadenopathy has a broad clinical spectrum that varies from spontaneous remissions to lethal infiltration of vital organs. There may be multiple extranodal sites of involvement that can be extremely aggressive in behavior, and virtually any organ in the body can be involved with the disease process.

Fourteen deaths have been reported in the SHML registry. These deaths do not seem to have any association with age, sex, or extranodal involvement. The most significant variable appears to be associated immunologic dysfunction.

There is an extensive differential diagnosis associated with SHML, but most other entities can be ruled out histologically, clinically, or by the inability to culture any infecting organisms. Malignant lymphoma can be excluded on clinical and pathologic grounds. Storage diseases can be excluded in view of the absence of any significant amount of abnormal lipid, carbohydrate, or mucopolysaccharide in the cytoplasm of histiocytes. Eosinophilic granuloma is morphologically similar to SHML, but the latter does not include hepatosplenomegaly or bony abnormalities.

Many features of SHML suggest an infectious cause and these include fever, leukocytosis, elevated erythrocyte sedimentation rate, local lymphadenopathy, and the usually self-limited nature of the disease. However, attempts to culture or otherwise isolate an infectious organism have been unsuccessful. Although rhinoscleroma is histologically similar to SHML and many cases of SHML had originally been diagnosed as rhinoscleroma, *Klebsiella rhinoscleromatis* could not be cultured from these patients. Salmonellosis is similar with respect to morphology of the histiocytes and lymphangiocytosis, but negative serologic tests and the lack of gastrointestinal involvement make a diagnosis of salmonellosis unlikely.

The Epstein-Barr virus plays an unknown role in this disease. Antibodies against the virus have been isolated in some patients, but the significance of such isolation is unknown.[10] Clearly, the diagnosis of both SHML and Rosai-Dorfman disease may be aided in the future by the advancement of cytogenetic and genotyping procedures.[9]

CONCLUSION

This paper highlights the clinical, histologic, and etiologic aspects of SHML and Rosai-Dorfman disease, and presents an unusual case report of a patient with isolated head and neck soft tissue manifestations. It is important for otolaryngologists, pathologists, and other physicians to be familiar with the head and neck manifestations of Rosai-Dorfman disease, especially in light of its atypical presentations.

ACKNOWLEDGMENT — The slides were reviewed by Dr J. Rosai, who determined that they in fact represented Rosai-Dorfman disease with osseous and cartilaginous involvement. He commented that the extreme degree of fibrosis found in this case is typical for extranodal sites.

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TRACTION DIVERTICULUM OF THE HYPOPHARYNX FOLLOWING ANTERIOR CERVICAL SPINE SURGERY

CASE REPORT AND REVIEW

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Pharyngeal complications due to anterior cervical spine surgery (ACSS) are not rare. We describe the case of a traction diverticulum of the hypopharynx after ACSS, a complication that has not yet been reported. We discuss the possible mechanism. A review of the complications of ACSS that are of interest to the otolaryngologist is included.

KEY WORDS — anterior cervical fusion, hypopharyngeal diverticulum, laryngeal nerve injury, surgical complications, traction diverticulum.

INTRODUCTION

The reports of Robinson and Smith,[1] Dereymaker et al,[2] and Cloward[3,4] in the late 1950s marked the beginning of the era of anterior cervical spine surgery (ACSS). This surgery was designed to meet the following goals[5]: reduction, decompression of nerve roots and/or spinal cord, and fixation for permanent stability. A large exposure of the cervical spine is provided through a right cervical incision and blunt dissection of the cleavage plane between the thyroid and the carotid sheath. After removal of the disk, the spinal canal is entered through a trephine hole, and a decompression of the spine and roots, removal of osteophytes, or debridement of a fractured vertebral body can be achieved. A bony fusion is accomplished by inserting a cylindric dowel bone graft into the trephine hole. The intervertebral body bone grafting can be coupled with stabilization by osteosynthetic plates and screws.

The operation has gained widespread acceptance in the treatment of degenerative disk disease.[4] In cervical spine trauma with ligamentous instability Cloward's operation does not always provide adequate immediate stabilization, and after realignment other techniques such as a posterior approach may be needed.[6]

However, in spite of the relative safety of ACSS, the possibility exists of postoperative morbidity specifically linked with the anterior approach. Some degree of injury to the pharyngoesophagus and to the larynx and trachea may occur, and probably the most common postoperative complaint is mild dysphagia and transient hoarseness.[3] More serious exposure injuries include perforation of the pharyngoesophagus or trachea and temporary or permanent damage to the recurrent nerve.[7]

We here describe a previously unreported complication of ACSS. A traction diverticulum of the hypopharynx developed 8 months after operation and was promoted by scarring between the dowel bone graft and the pharyngoesophagus region. We then review the complications of ACSS of interest to the otolaryngologist.

CASE REPORT

In October 1987 a 44-year-old man sustained a motor vehicle accident and was brought to the hospital. Radiographs showed a C-6 and C-7 fracture/dislocation. No immediate neurologic deficit was noted, but gradual left arm paresis appeared. An attempt at reduction in the emergency room with the patient awake on a Stryker frame using skull tongs traction failed. After induction of general anesthesia, reduction was obtained. An anterior approach was decided on and was carried out through a right incision. At operation the intervertebral disk was ruptured, with a fragment lodged in the spinal canal. After debridement an 18-mm bone dowel was impacted. Postoperatively, a Schanz collar was used for external stabilization.

The initial postoperative status was normal, but on the ninth postoperative day the patient complained of pain in the neck irradiating to the forearms and hands. A few hours later severe neurologic deficits developed in the upper extremities. Radiographs showed a ventral displacement of the graft that required immediate surgical replacement. After removal of the initial graft a new, well-fitting cylindric bone graft was inserted. Postoperatively, a halo apparatus was used until 3 months after operation. The radiculomyelopathy gradually improved, with residual weakness of the hands and spasticity in the lower extremities. As the radio-

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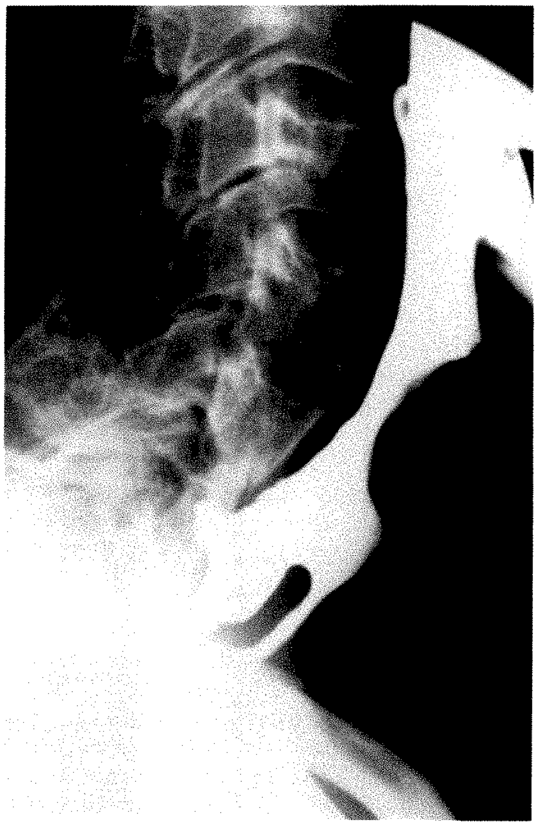

Barium swallow study in patient with hypopharyngeal diverticulum. Note encroachment on bone graft.

graphs showed an adequate fusion, a Schanz collar was then used for 3 more months.

In June 1988 progressive dysphagia and regurgitation of food developed and resulted in considerable weight loss (15 kg over a 3-month period). In September 1988 an otolaryngologic consultation was requested. Findings on physical examination were unremarkable except for pooling of saliva in the pyriform sinuses. Lateral radiographs demonstrated complete interbody fusion of C-6 and C-7 with partial resorption of the graft. Air was readily visible behind the pharyngoesophageal junction. A cineradiographic swallowing study revealed a hypopharyngeal diverticulum coming in close contact with the bone graft (see Figure). The cricopharyngeus muscle relaxed appropriately. At operation the hypopharyngeal diverticulum was found to be very adherent to the bone graft, which appeared slightly resorbed. After elevation and identification of its neck, the diverticulum was clamped and excised. The recurrent laryngeal nerve could not be positively identified. A cricopharyngeal myotomy was performed. A piece of fascia lata was secured in front of the cervical spine to reconstruct the prevertebral fascia. A nasogastric tube was used for feedings for the first 6 postoperative days. Postoperatively, right recurrent nerve palsy was noted. The patient resumed near-normal swallowing. (Eighteen months after operation he experienced occasional sticking of food.) Over a period of a few weeks his voice strength returned.

DISCUSSION

Since the beginning of ACSS, specific morbidity and mortality related to the exposure have arisen, although major or long-lasting complications are rare. As these complications affect the deep structures of the neck, the otolaryngologist is often involved in evaluation and treatment.

Our case was a delayed complication of ACSS. We describe the unusual radiographic and surgical presentation of a hypopharyngeal diverticulum with symptoms appearing 8 months after operation. To our knowledge this is the first documented case of a traction diverticulum of the hypopharynx after ACSS.

The classic hypopharyngeal diverticulum, or Zenker's diverticulum, is a herniation of the hypopharyngeal mucous membrane in the locus minor resistentiae between the muscle fibers in the upper esophageal sphincter region.[8] In spite of the advent of manometric methods, opinions concerning its pathophysiology are somewhat controversial, but the most popular theory remains that the outpouching of the pharyngeal wall is the result of increased pressure in the pharynx during swallowing, secondary to a dysfunction of the cricopharyngeus muscle. This concept led to the term pulsion diverticulum.

A traction diverticulum, on the other hand, results from pulling of the esophageal wall, usually due to an inflammatory process in the surrounding area. Hypopharyngeal traction diverticula are extremely rare and were described in 1840 by Rokitansky (cited by Duda et al[9]). A case has been reported secondary to a cervical esophagostomy by Kaplan et al.[10] Decades ago, midesophageal diverticula were classically considered to be due to external traction from inflamed mediastinal lymph nodes in tuberculosis,[11] but this condition has become almost nonexistent in Europe.

In this case the history and the radiographic and surgical findings support the hypothesis that the development of the diverticulum was promoted by an adhesion between the hypopharynx and the bone graft. Swallowing and continued peristalsis of the pharyngoesophagus created a shearing stress around a fixed pharyngeal adhesion and led after a few months to a symptomatic diverticulum. The reoperation on the ninth postoperative day — probably due to inadequate stabilization of the spine and subsequent dislodgment of the graft[12] — might have been the promoting factor for adherences between the bone graft and the hypopharynx. The partial resorption of the bone dowel, visible in the Figure, might have created a well for the pharyngoesophagus to herniate into. Therefore, this should be thought of as a traction diverticulum.

The diverticulum was successfully managed by excision. Although cricopharyngeal dysfunction was not of concern, it seemed wisest to perform a cricopharyngeal myotomy, since the added operating time and morbidity are insignificant. According to the presumed pathophysiology of the diverticulum, a reconstruction of the prevertebral fascia with a piece of fascia lata ended the procedure.

As in other operations in the lower part of the neck, various pharyngeal and laryngeal complications have been reported. It is noteworthy that the complications previously reported are immediate in the postoperative course, and the diagnosis is usually straightforward.

Difficulty in swallowing is the most common complaint after ACSS. The dysphagia is probably caused by postoperative edema of the esophagus due to the spreading retractors.[3] This symptom usually disappears within 48 hours. Bulger et al[13] reported a 16.7% incidence of significant postoperative dysphagia in a retrospective study of 102 patients. They suggest that mild dysphagia should not be regarded as particularly worrisome unless it lasts more than 6 weeks. Long-lasting swallowing problems, defined as being present at 3 months' follow-up but unfortunately of an unspecified nature, are reported in 9 patients by Espersen et al[14] in a survey of 1,106 cases (0.9%).

The actual incidence of perforation of the hypopharynx or esophagus following ACSS is not known, as the literature regarding ACSS has primarily focused on neurologic complications. Capen et al[15] reported 2 cases of esophageal trauma requiring reoperation for drainage of abscess and nasogastric tube feedings in a series of 88 patients who had undergone ACSS. Flynn[16] surveyed 704 neurosurgeons by questionnaire and reported, of a total of 82,114 patients who had ACSS, 5 patients who succumbed to pharyngeal perforation. Balmaseda and Pellioni[17] reported 1 case of esophagocutaneous fistula successfully managed by conservative treatment. In most circumstances perforation of the pharyngoesophagus has been attributed to penetration by the sharp blades of a spreading retractor. In some cases osteosynthetic material was responsible for dysphagia. One case of perforation of the hypopharynx by a loosened screw has been reported.[18]

Damage to the trachea, larynx, or recurrent nerve is also a possible complication of ACSS. Transient hoarseness is quite common after ACSS. Temporary hoarseness may be due to recurrent nerve trauma, but frequently is the result of swelling in the soft tissues of the pharynx and larynx. Death from laryngeal edema has been reported.[14]

No systematic laryngeal examination of a series of patients has been carried out, and the actual incidence of hoarseness resulting from permanent vocal cord palsy is not known, but the frequency ranges from 0.06% to 4%. Flynn[16] noticed only 52 cases of recurrent nerve palsy in his survey of 82,114 patients with ACSS (0.06%). Espersen et al[14] reported 6 transient and 6 persistent laryngeal paralysis cases in 1,106 patients with ACSS (0.6%). Bulger et al[13] reported a 21.6% incidence of hoarseness in a retrospective study of 102 patients with ACSS out of a series of 375 patients, but only 1 patient was found having persistent recurrent nerve palsy (1%), and another had superior laryngeal nerve palsy. Dohn[19] reports in his series of 210 patients 3 cases of proven vocal cord palsy (1.5%) among 7 hoarse patients. Heeneman[20] reviewed the charts of 85 patients with ACSS. Six patients were found to have transient impairment of laryngeal mobility, and 3 (4%) did not recover.

According to its presumed incidence, recurrent nerve palsy could be related, at least partly, to a rare anatomic variant (approximately 0.5% to 1% of cases) in which the inferior laryngeal nerve is found to be nonrecurrent.[21] During ACSS, the nonrecurrent nerve is fully exposed to surgical trauma during dissection of the areolar tissue between the carotid sheath and the right thyroid lobe.

It is also known that true vocal cord palsy following intubation may occur,[22] and it has been related by Cavo[23] to a compression by the endotracheal tube of the anterior branch of the recurrent laryngeal nerve in a submucous area situated 6 to 10 mm below the level of the true vocal cords. It is possible that during ACSS, retraction of the larynx may increase the pressure of a high-placed endotracheal tube in that specific area. Excessive retraction could also cause a shearing action of the cricoid cartilage in relation to the thyroid cartilage and result in an injury to the recurrent laryngeal nerve. These last two mechanisms could account especially for transient laryngeal paralysis observed after ACSS.

CONCLUSION

We present the case of a traction diverticulum of the hypopharynx following ACSS successfully managed by excision, cricopharyngeal myotomy, and fascia lata interposition. We review the literature concerning otolaryngologic complications of ACSS. It appears that their frequency, although difficult to establish, makes them noticeable to the otolaryngologist. We suggest that in swallowing problems persisting or appearing after ACSS the patient be considered for cineradiographic studies as various pharyngeal complications may be encountered.

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DIAGNOSTIC AND OPERATIVE SINUS ENDOSCOPY

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TRACHEOMALACIA AND BRONCHOPULMONARY DYSPLASIA

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Wheezing in the infant with bronchopulmonary dysplasia results from various pathophysiologic mechanisms, including upper or central airway obstruction. We report an infant with bronchopulmonary dysplasia who presented with episodic wheezing associated with hypoxia and bradycardia without evidence of stridor. Bronchoscopy revealed the presence of tracheomalacia. Upper or central airway obstruction, if undiagnosed, may be life-threatening. We suggest that wheezing with hypoxia and bradycardia in neonates should alert the physician to the possibility of tracheomalacia, even in the absence of audible stridor. Infants presenting with these symptoms should undergo bronchoscopy to confirm the diagnosis.

KEY WORDS — bronchopulmonary dysplasia, central airway obstruction, neonates, tracheomalacia.

INTRODUCTION

Infants on assisted ventilation for long periods are subject to injury of the upper airway, respiratory tree, and lungs. Injuries suffered during prolonged assisted ventilation include pneumothorax, pulmonary interstitial emphysema, and the development of bronchopulmonary dysplasia (BPD). Less well-known injuries to the respiratory tree resulting from prolonged endotracheal intubation include laryngeal stenosis, subglottic stenosis, tracheal stenosis, and tracheitis.[1-4]

Some of these entities may exist unrecognized in the neonatal population with BPD. When stridor is present, laryngeal or tracheal disease can be suspected. However, when hypoxia and blue spells, which are common in infants with BPD, are the only clinical manifestations, the diagnosis of upper or central airway disease can challenge the clinical acumen of neonatologists and pulmonologists alike. Indeed, patients with known BPD may exhibit episodes, similar to anoxic spells, that may be directly related to tracheomalacia. We report on an infant with BPD and tracheomalacia and review the associated disease states and pathologic findings, including the abnormalities noted on pulmonary function testing.

CASE REPORT

The patient, the first of twins, was born at 36 weeks' gestation to a 34-year-old woman (gravida 5, para 3, aborta 3) via cesarean section for partial abruptio placentae. His Apgar scores were 1 at 1 minute and 8 at 5 minutes. His weight, length, and head circumference were appropriate for his gestational age. He required 27 days of assisted ventilation for respiratory failure that was secondary to acute perinatal blood loss.

Following extubation, the infant developed recurrent episodes of wheezing associated with irritability, bradycardia, and hypoxia. These episodes occurred two to five times per week and were noted with coughing, feeding, and the passing of stool or flatus. His response to bronchodilators and sedation was inconsistent. Control of gastroesophageal reflux did not alleviate the symptoms.

Physical examination during an episode typically revealed tachypnea; suprasternal, intercostal, and subcostal retractions; inspiratory and expiratory wheezing; and poor air exchange. A chest radiograph revealed hyperinflation with chronic lung disease. A barium contrast study of the upper gastrointestinal tract revealed gastroesophageal reflux, but no evidence of extrinsic compression. Results of a sleep study were normal. Pulmonary function testing (SensorMedics system 2600, Yorba Linda, Calif) revealed intrathoracic obstruction with reduction of the midexpiratory-to-midinspiratory ratio on the flow-volume loop compatible with tracheomalacia. A laryngoscopic examination revealed no abnormalities. Flexible bronchoscopy confirmed the anterior-posterior collapse of the distal third of the trachea, with lengthening and ballooning of the pars membranacea during expiration. The cartilaginous rings were flattened in this area. Structures in the distal trachea and bronchi were normal in appearance. To further confirm that tracheomalacia was the cause for the recurrent episodes, elective endotracheal intubation was performed and maintained for a period of 5 days, during which no episodes of wheezing, bradycardia, or hypoxia occurred.

DISCUSSION

Congenital and acquired tracheomalacia have been associated with a variety of anomalies. These conditions can be classified into two major groups: chronic irritation or inflammation and anatomic abnormalities. Chronic inflammation may result both from acquired and congenital conditions. Most acquired tracheomalacia is iatrogenic, resulting from tracheostomy or cuffed endotracheal tubes.[5] Further, assisted ventilation and chronic obstruc-

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tive pulmonary disease may result in tracheomalacia.[6] The inflammatory conditions of polychondritis[7] and Larsen's multiple joint syndrome[8] may result in congenital tracheomalacia. Anatomic abnormalities that compress the trachea may produce tracheomalacia, and include multiple cardiovascular anomalies (double aortic arch and anomalous innominate artery), bronchogenic or other duplication cyst, teratoma, abscess, cystic hygroma, and hemangioma.[9,10] Other anomalies have been associated with tracheomalacia, most frequently tracheoesophageal fistula.[10-12] Our patient did not have any extrinsic compression, and no congenital anomalies.

Tracheal dynamics in normal and abnormal infants, as well as in experimental conditions, have been studied by Wittenborg et al.[13] In the normal neonate, the anterior-posterior luminal diameter varies by 20% to 50% during forced inspiratory or expiratory efforts. In abnormal infants displaying stridor or other symptoms of respiratory distress, tracheal lumen changes are greater, and inspiratory and expiratory tracheal collapse can be demonstrated. These patterns of collapse reflect an increased transmural pressure resulting from a respiratory effort produced to overcome increased airway resistance. Brief inspiratory collapse occurs in a short segment of the trachea at the cervicothoracic junction. Experimentally, inspiratory collapse can be reproduced by a fixed subglottic obstruction. Prolonged expiratory collapse during the late phases of expiration affects the intrathoracic trachea. This type of collapse is common in obstructive lesions of the peripheral airway, such as that seen in asthma and bronchiolitis. Experimentally, this type of obstruction can be reproduced by anatomic obstruction at the carina, or by methacholine, resulting in bronchiolar obstruction.[13]

Infants with BPD are at great risk for the development of tracheomalacia. This results from airway injury due to prolonged mechanical ventilation, barotrauma, oxygen toxicity, pulmonary infections, and mucociliary abnormalities. As noted above, peripheral airway obstruction plays a role in tracheal dynamics resulting in expiratory collapse of the airway. Indeed, in the adult population with chronic obstructive pulmonary disease (COPD), the severity of COPD parallels the degree of tracheomalacia, and with the presence of tracheomalacia, there is an accelerated progression of COPD.[6] Further, the presence of additional airway anomalies common to infants with BPD (eg, laryngeal stenosis, subglottic stenosis) may cause obstructive phenomena within the airway and contribute to the production and/or the progression of tracheomalacia. Miller et al[14] performed bronchoscopy on 12 infants with bronchopulmonary dysplasia. Findings included granulation tissue resulting in partial to total airway obstruction (10 patients), inspissated secretions (2 patients), and tracheomalacia, bronchomalacia, or both (3 patients). Of the 3 patients with tracheomalacia or bronchomalacia, one had granulation tissue at the carina, while one had inspissated mucous casts. All 3 died before 7 months of age. This finding underscores the importance of airway anomalies in infants with BPD, and suggests that tracheomalacia may be more common than previously thought.

The diagnosis of tracheomalacia may be suggested by clinical signs, radiographic examination, and pulmonary function testing. However, bronchoscopic evaluation should always be performed to confirm suspicion. Classic signs include cough, respiratory distress (manifested by tachypnea, flaring, grunting, and retractions), and inspiratory stridor. However, signs suggestive of obstructive apnea may predominate, as illustrated in our case, and include respiratory distress, bradycardia, and hypoxia. Stridor is not an absolute finding in patients with BPD and tracheomalacia.[15] Radiographic evaluations may show additional evidence of tracheomalacia. Fluoroscopic examination with cineradiography may reveal tracheal narrowing or collapse on lateral view. However, the absence of radiographic findings does not exclude the diagnosis. Flow limitations on the expiratory component of the flow-volume loop with a decreased midexpiratory-to-midinspiratory ratio suggest the diagnosis of tracheomalacia.[16] Findings during bronchoscopy include a lengthened pars membranacea, flattened or distorted cartilaginous rings, and anterior-posterior collapse of the tracheal lumen. A delay in diagnosis is not uncommon. In a series of seven infants with chronic lung disease and tracheomalacia, Sotomayor et al[15] showed a mean delay of 6 months from the onset of symptoms to the time of diagnosis.

The natural history of tracheomalacia in the neonate with BPD is poorly defined. Eight preterm infants with BPD and tracheomalacia and/or bronchomalacia have been previously described.[14,15] These infants underwent bronchoscopy for persistent respiratory abnormalities, including signs suggestive of obstructive apnea. All infants required long-term ventilatory support in the form of intermittent mandatory ventilation or continuous positive airway pressure. Three of these patients died by 7 months of age. Despite this poor outcome, some of these patients will improve and become asymptomatic over time. Optimal management of BPD is necessary. Additionally, aggressive management of infections involving the respiratory tree is essential. Minimizing correctable airway anomalies may lessen the severity and/or progression of tracheomalacia. Additional airway management may be necessary, particularly in the patient who develops life-threatening airway occlusion due to tracheomalacia.[15,17,18] This often can be accomplished by endotracheal intubation, tracheostomy, or with the addition of high levels of continuous positive airway pressure or positive end-expiratory pressure. These patients may require levels as high as 10 to 20 cm H_2O in order to maintain patency of the tracheal lumen.[15] Surgical intervention appears useful only in cases of segmental collapse.

CONCLUSION

In the typical infant with BPD, wheezing and hypoxia are often attributed to bronchospasm. A high index of suspicion is required to discover the presence of tracheomalacia. In an infant with wheezing, hypoxia, and bradycardia, pulmonary function testing and examination of the airway should be undertaken to demonstrate abnormalities. Stridor is not necessarily part of the complex of symptoms. A multidisciplinary approach to management should be employed, with attention to airway patency, control of small airway disease, and eradication of infections. Only through examination of those infants at risk will tracheomalacia be diagnosed, the incidence determined, and the clinical characteristics and course defined.

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TRANSPORT OF 5-HYDROXYDOPAMINE AND HORSERADISH PEROXIDASE THROUGH THE PERILYMPH-ENDOLYMPH BARRIER

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The distribution of 5-hydroxydopamine (5-OHDA) and horseradish peroxidase (HRP) in the cochlea was examined by light and electron microscopy after perilymphatic perfusion. Reaction product of 5-OHDA was observed along the endolymphatic surface of the cochlear duct in both 10-minute and 30-minute perfusions, while that of HRP was observed only in the 30-minute perfusion. Both tracers were found in pinocytotic vesicles of Reissner's membrane, Claudius' cells, and Hensen's cells. No labeling was seen in tight junctions of the epithelial cells lining the scala media. The epithelial cells of the spiral prominence showed various degrees of degeneration in the 5-OHDA experiment. These morphologic findings suggest that 5-OHDA and HRP can enter the endolymphatic space from the perilymphatic space by pinocytosis and that the degree of transport depends on the size of the particles.

KEY WORDS — horseradish peroxidase, 5-hydroxydopamine, perilymph-endolymph barrier, tight junctions.

INTRODUCTION

It is well known that chronic otitis media induces sensorineural hearing loss because of chemical mediators and toxins permeating the round window membrane into the perilymph and then penetrating into the endolymph. Several studies clarified the pathway through the round window membrane by using morphologic tracers, ie, transcellular transport by pinocytosis.[1-3] However, the pathway from the perilymph to the endolymph has not been well established.

It has been proposed that there exists a barrier between the perilymph and the endolymph.[4-6] Electron-dense tracers have been used to examine the permeability of the barrier. It has been shown that horseradish peroxidase (HRP) introduced into the perilymph was not transported to the endolymphatic space owing to the tight junctions between the epithelial cells lining the endolymphatic surface.[3,7,8] However, ferritin injected into the scala vestibuli entered the scala media through Reissner's membrane.[9] So far, the permeability of this barrier has been discussed only with respect to macromolecules such as HRP (molecular weight, 40,000; diameter, 5 nm) and ferritin (molecular weight, 465,000; diameter, 5 to 10 nm).

Physiologically, perilymphatic perfusion of loop diuretics such as furosemide (molecular weight, 330) and ethacrynic acid (molecular weight, 303) decreases the endocochlear potential.[10,11] This finding suggests the possibility that smaller molecular substances may gain access to intercellular spaces of the stria vascularis, in which the endocochlear potential is thought to be produced.

5-Hydroxydopamine (5-OHDA) is known to be a specific marker for monoaminergic nerve terminals. It also has tracer properties similar to those of HRP.[12,13] To our knowledge, it is one of the smallest tracers (molecular weight, 256; diameter, 0.5 to 0.7 nm) that has a reaction product visible by transmission electron microscopy.

The purposes of the present study are to examine the ultrastructural distribution of 5-OHDA and HRP in the cochlea and to clarify the mode of perilymph-endolymph transport.

MATERIALS AND METHODS

A total of 22 chinchillas weighing 430 to 670 g were purchased from a single supplier and kept on a standard diet. Animals were anesthetized with an intramuscular injection of ketamine hydrochloride (40 mg/kg), and they were artificially respirated through the tracheal cannula after an intramuscular injection of gallamine triethiodine (8 mg/kg). The tympanic bulla was exposed and a small hole for the perfusion was made in the scala tympani of the basal turn. The stapes was subluxated as an outlet.

5-Hydroxydopamine (Aldrich Chemical Co) and

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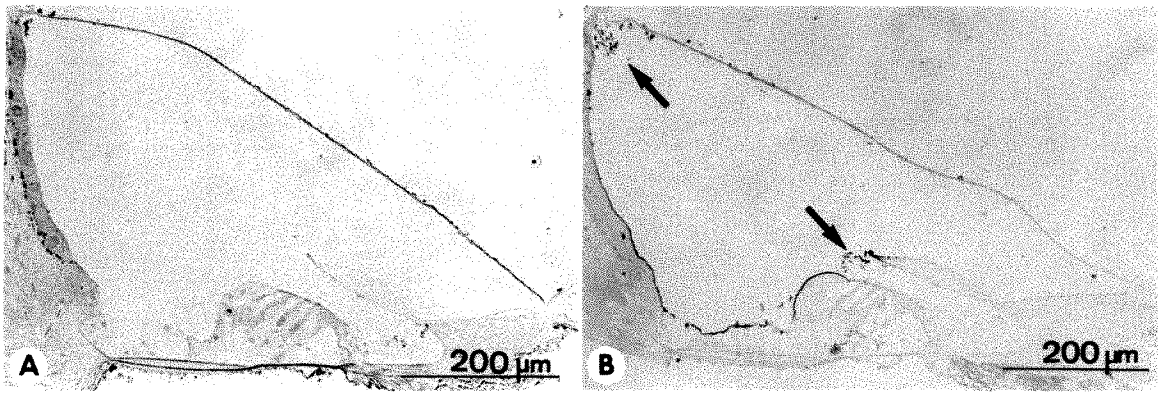

Fig 1. Light micrographs of cochlear duct in 5-hydroxydopamine experiment. Three-micron-thick, unstained sections of second turn. A) Ten-minute perilymphatic perfusion. Reissner's membrane and mesothelial cells of basilar membrane are stained. B) Thirty-minute perilymphatic perfusion. Endolymphatic surface of epithelial cells lining scala media is densely stained except for inner sulcus cells and interdental cells. Deposits of reaction product are observed in scala media (arrows).

HRP (type II, Sigma Co) were used as tracers. The molecular weight and molecular diameter were estimated to be 256 and 0.5 to 0.7 nm, respectively, for 5-OHDA, and 40,000 and 5 nm for HRP. Tracers were dissolved in the artificial perilymph, which consisted of KCl 3.5 mmol/L, NaCl 125 mmol/L, $NaHCO_3$ 25 mmol/L, $CaCl_2$ 1.3 mmol/L, $MgCl_2$ 1 mmol/L, NaH_2PO_4 0.75 mmol/L, glucose 3 mmol/L, and urea 2 mmol/L. The pH was adjusted to 7.4 by bubbling with a gas mixture of 95% O_2 and 5% CO_2.[14]

5-HYDROXYDOPAMINE

To avoid an increase in intraperilymphatic pressure, a 1% solution of 5-OHDA was perfused very slowly (10 µL/min) into the perilymph by using a micropipet and an infusion pump. Two different perfusion times were used: 10 minutes (n = 3) and 30 minutes (n = 5). After perfusion of the tracer, cochleas were perilymphatically perfused with 2% ice-cold glutaraldehyde in 0.1 mol/L sodium chromate–potassium dichromate buffer, pH 7.2, for 10 minutes. The whole cochleas were removed from the skull, and the stapes and a small part of the bone at the apex was removed. While the cochleas were immersed on a rotator in the same fixative for 1 hour, the fixatives were perfused by a small syringe from the round window to the apex every 15 minutes. Thereafter, the whole cochleas were placed in 0.2 mol/L cold sodium chromate–potassium dichromate buffer, pH 6.0, for 18 hours and postfixed for 2 hours in 1% cold OsO_4 in sodium chromate–potassium dichromate buffer, pH 7.2.[12]

HORSERADISH PEROXIDASE

Two different perfusion times were used: 10 minutes (n = 4) and 30 minutes (n = 6). After perilymphatic perfusion of a 1% solution of HRP, the cochleas were perfused for 10 minutes with 2% ice-cold glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.3, and then immersed for 30 minutes in the same fixative. The whole cochleas were removed from the skull and were preincubated with ice-cold 0.05 mol/L Tris buffer, pH 7.6, plus 150 mg Hanker-Yates incubation media (100 mL Tris buffer, pH 7.6, plus 150 mg Hanker-Yates reagent plus 0.1 mL of 30% H_2O_2) at room temperature.[15] During the preincubation and incubation, the whole cochleas were perfused with the incubation medium by a small syringe from the round window to the apex every 15 minutes. They were then postfixed for 1 hour in 1% OsO_4 in phosphate buffer.

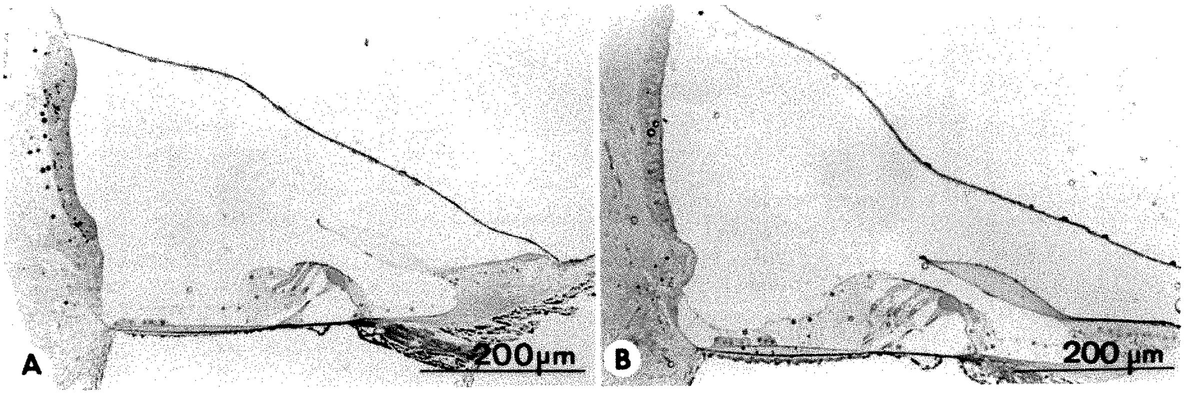

Fig 2. Light micrographs of cochlear duct in horseradish peroxidase (HRP) experiment. Second turn. A) Ten-minute perilymphatic perfusion. Endolymphatic surface of epithelial cells lining scala media was not stained with HRP. B) Thirty-minute perilymphatic perfusion. Reaction product was observed in scala media near epithelial surface.

NUMBER OF ANIMALS SHOWING TRACER IN SCALA MEDIA AT MICROSCOPIC LEVEL IN EACH GROUP

	10-Minute Perfusion	*30-Minute Perfusion*
5-Hydroxydopamine	3/3	5/5
Horseradish peroxidase	0/3	2/5

CONTROL STUDY

The same amounts of artificial perilymph were perfused in the perilymph for 30 minutes instead of 5-OHDA (n = 2) or HRP (n = 2). The demonstration of the reaction product of either 5-OHDA or HRP was performed in the same way as mentioned above.

The cochleas were not decalcified. They were dehydrated in a series of ethyl alcohols and embedded in methyl methacrylate (Medcast). Three-micrometer-thick cross sections of the whole scala media were cut from each of the three turns of each cochlea. They were left unstained and were examined by light microscopy. Ultrathin sections for electron microscopy were stained with either uranyl acetate and lead citrate or with uranyl acetate only, and examined on a JEOL 100S electron microscope.

RESULTS

LIGHT MICROSCOPY

In the 10-minute perfusion of 5-OHDA, the endolymphatic surface of cells lining the scala media was slightly stained, and reaction product was barely detectable in the scala media at the light microscopic level (Fig 1A). In the 30-minute perfusion, the endolymphatic surface of the cells lining the scala media was densely stained. Clusters of the reaction product were sometimes found in the scala media (Fig 1B).

In the 10-minute perfusion of HRP, the endolymphatic surface of the cochlear duct was not stained (Fig 2A). In the 30-minute perfusion of HRP, reaction product of HRP was observed close to the surface of the epithelial cells (Fig 2B).

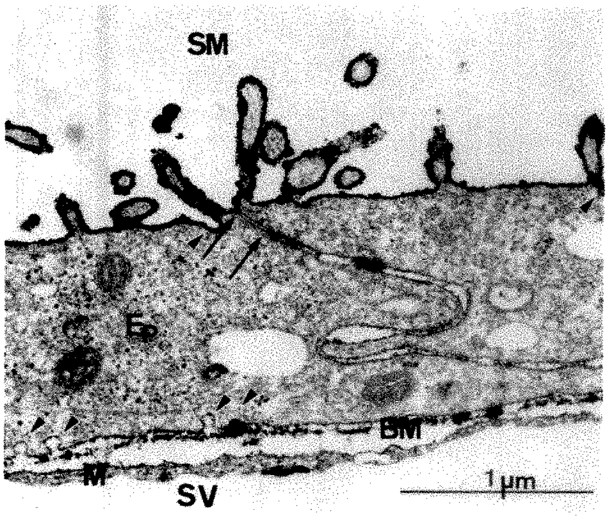

Fig 3. Electron micrograph of Reissner's membrane after 30-minute perfusion of 5-hydroxydopamine (5-OHDA). Third turn. Endolymphatic surface of epithelial cells (Ep) is densely stained with reaction product of 5-OHDA. Basement membrane (BM) and mesothelial cells (M) on scala vestibuli (SV) are also stained. No reaction product is detected at tight junctions of Ep (between arrows) facing scala media (SM). Many labeled pinocytotic vesicles, some of which are open to SM and some to SV, are observed (arrowheads).

The Table summarizes the results of the light microscopic observations mentioned above.

ELECTRON MICROSCOPY

5-Hydroxydopamine. The perilymphatic surface of Reissner's membrane was densely stained with reaction product in both 10-minute and 30-minute

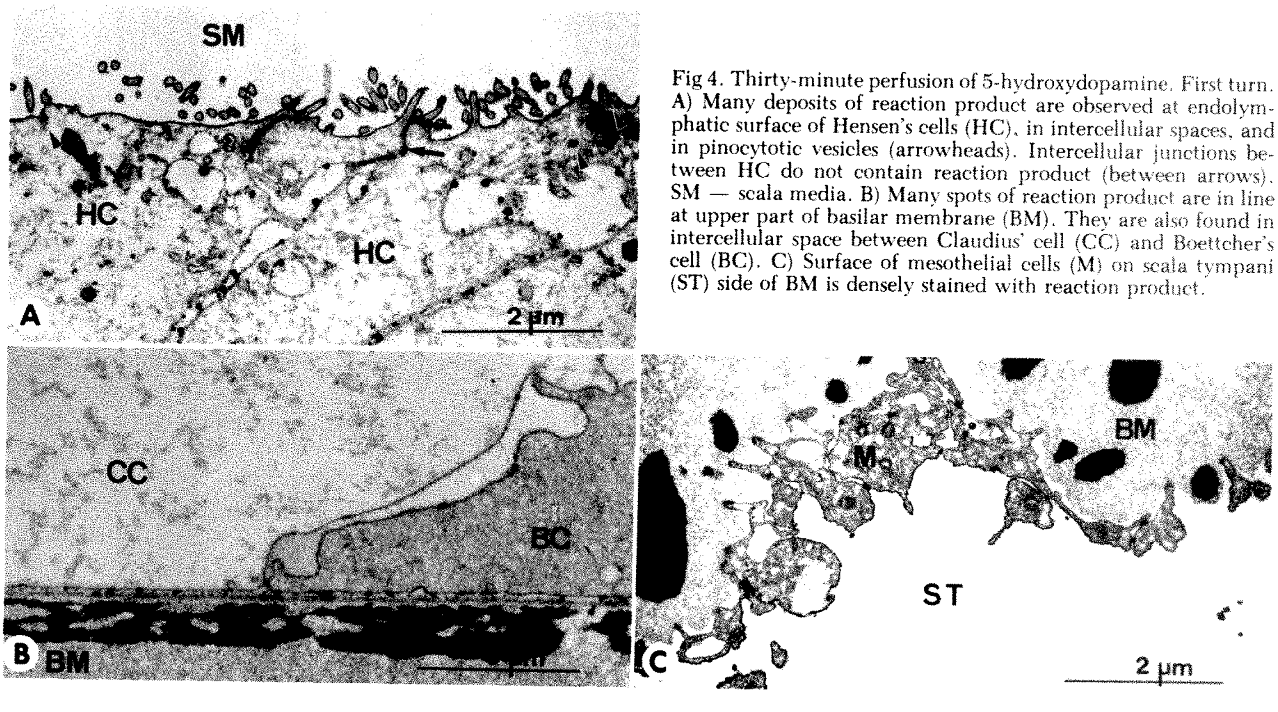

Fig 4. Thirty-minute perfusion of 5-hydroxydopamine. First turn. A) Many deposits of reaction product are observed at endolymphatic surface of Hensen's cells (HC), in intercellular spaces, and in pinocytotic vesicles (arrowheads). Intercellular junctions between HC do not contain reaction product (between arrows). SM — scala media. B) Many spots of reaction product are in line at upper part of basilar membrane (BM). They are also found in intercellular space between Claudius' cell (CC) and Boettcher's cell (BC). C) Surface of mesothelial cells (M) on scala tympani (ST) side of BM is densely stained with reaction product.

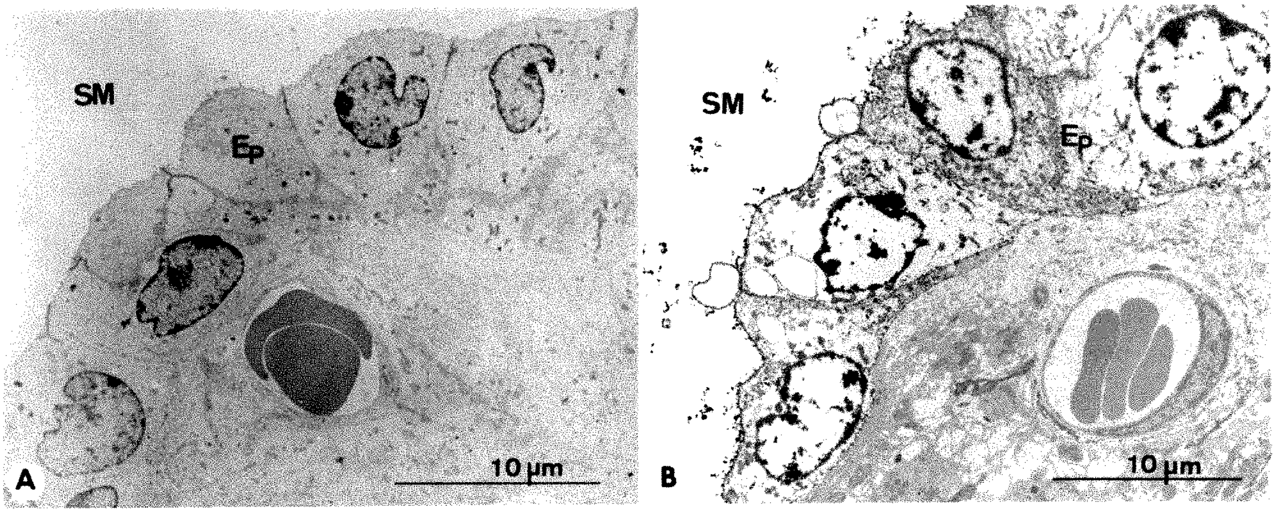

Fig 5. Epithelial cells (Ep) of spiral prominence. First turn. SM — scala media. A) Thirty-minute perfusion of artificial perilymph only instead of 5-hydroxydopamine (5-OHDA) solution. Epithelial cells are intact. B) Thirty-minute perfusion of 5-OHDA. Much reaction product is observed in SM and on surface of Ep. Cytoplasm of Ep is sparse. Some vacuoles are found in cytoplasm and others protrude in SM. However, neighboring cells are intact.

experiments. The endolymphatic surface was more densely stained in the 30-minute experiment than in the 10-minute experiment. Epithelial cells of Reissner's membrane showed a number of labeled pinocytotic vesicles, but did not contain the reaction product in the tight junctions (Fig 3).

The endolymphatic surface of Claudius' cells and Hensen's cells was densely stained. Numerous deposits of reaction product, which were round in most cases, were attached to the cell membrane along the intercellular spaces between Claudius' cells and between Hensen's cells (Fig 4A), and along Nuel's space and the tunnel of Corti. 5-Hydroxydopamine was also observed in pinocytotic vesicles (Fig 4A). Tracer substances were located in the basilar membrane, especially in the area close to epithelial cells (Fig 4B). The perilymphatic surface of mesothelial cells of the basilar membrane was also densely stained (Fig 4C).

The epithelial cells of the spiral prominence were intact in the control (Fig 5A) and 10-minute experiments. On the other hand, they showed various degrees of degeneration in the 30-minute experiment. The other cells of the spiral prominence and external sulcus were intact (Fig 5B).

The endolymphatic surface of the stria vascularis was stained in both experiments. The degree of staining was higher in the 30-minute experiment than in the 10-minute experiment. No reaction product was found inside the stria vascularis (Fig 6), while it was sometimes found in the intercellular spaces of the spiral ligament.

In the organ of Corti, reaction product of 5-OHDA was distributed on the lateral surface of hair cells and Deiters' cells (Fig 7A). Of interest, there was an accumulation of tracer substances in the synaptic gap (Fig 7B).

Horseradish Peroxidase. The perilymphatic surface of Reissner's membrane was densely stained with reaction product in both 10-minute and 30-minute experiments. The endolymphatic surface was not stained in the 10-minute experiment, but was stained in the 30-minute experiment. Epithelial cells of Reissner's membrane showed many micropinocytotic vesicles and did not show the tracer in tight junctions (Fig 8).

A number of fuzzy dots with a core, which is considered to include reaction product, were observed throughout the basilar membrane, Claudius' cells, and Hensen's cells (Fig 9).

Horseradish peroxidase was observed at the endolymphatic surface of the stria vascularis, but it was not observed inside the stria vascularis.

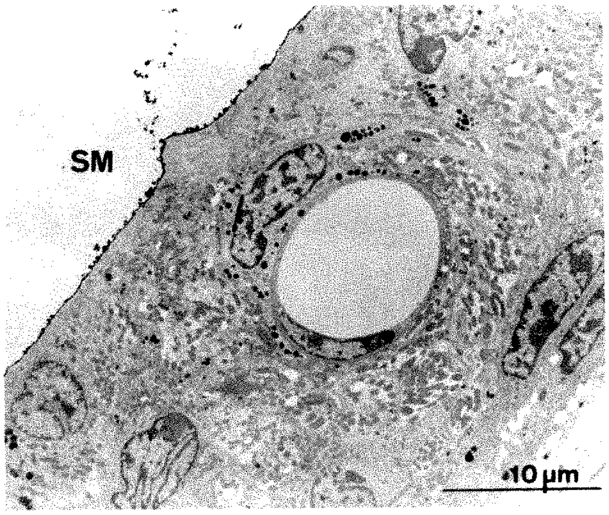

Fig 6. Stria vascularis after 30-minute perfusion of 5-hydroxydopamine. Second turn. Endolymphatic surface is densely stained with tracer. No reaction product is found in stria vascularis. SM — scala media.

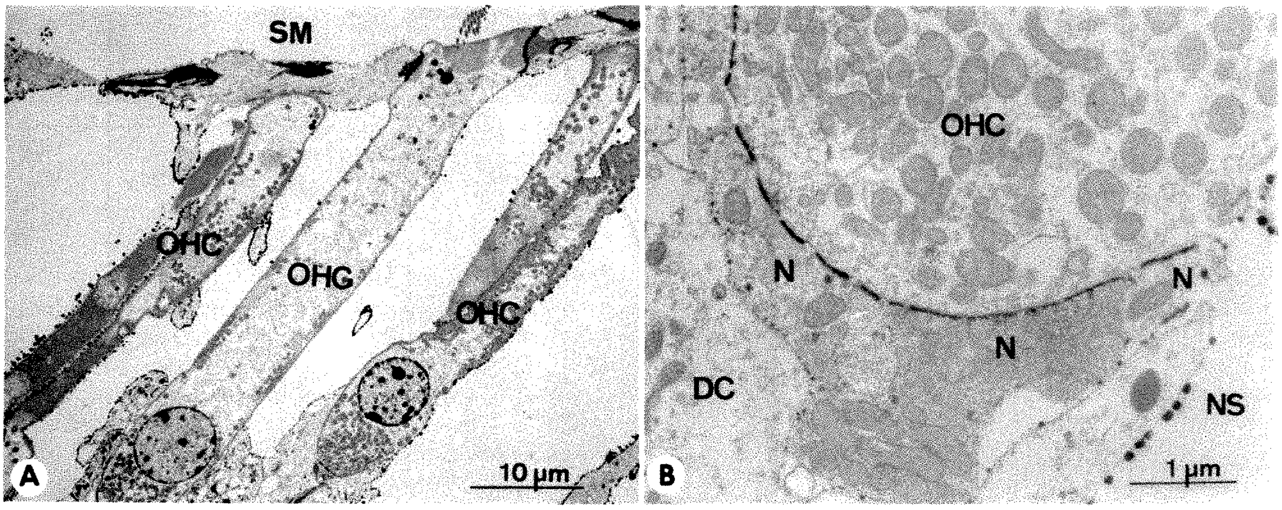

Fig 7. Outer hair cells (OHC) after 10-minute perfusion of 5-hydroxydopamine. Second turn. A) Reaction product is observed on lateral surface of OHC. SM — scala media. B) Higher magnification of basal portion of one OHC. Reaction product fills synaptic gaps between OHC and nerve endings (N). DC — Deiters' cell, NS — Nuel's space.

Control Study. The cell structure of the cochlear duct was not damaged by the perfusion with artificial perilymph only (Fig 5A). No endogenous 5-OHDA activity was found in the cochlea. Endogenous peroxidase activity was not observed except in lysosomes.

DISCUSSION

5-Hydroxydopamine, a noradrenergic analogue, has been used as a specific marker for adrenergic terminals. There have been studies of cytochemical localization of neuronal storage sites of 5-OHDA in the brain[16-18] and in the inner ear.[19] During those experiments it was discovered that highly electron-dense material accumulated in the extracellular space of the brain after intraventricular perfusion. This observation suggested that 5-OHDA, a comparatively small molecule (molecular weight, 256; diameter, 0.5 to 0.7 nm), exhibited tracer properties similar to those of peroxidase.[12,13]

In the present study, the endolymphatic surface of epithelial cells lining the scala media was stained with reaction product of 5-OHDA even after 10-minute perilymphatic perfusion; however, the degree of staining was less in 10-minute perilymphatic perfusion than in 30-minute perfusion. These findings suggest that the entry of 5-OHDA from the perilymph to the endolymph increased in relation to the perfusion time.

Previous experiments showed that HRP (molecular weight, 40,000; diameter, 5 nm) did not enter the endolymph, at least during the short perfusion time applied in the present study.[3,7,8] However, the

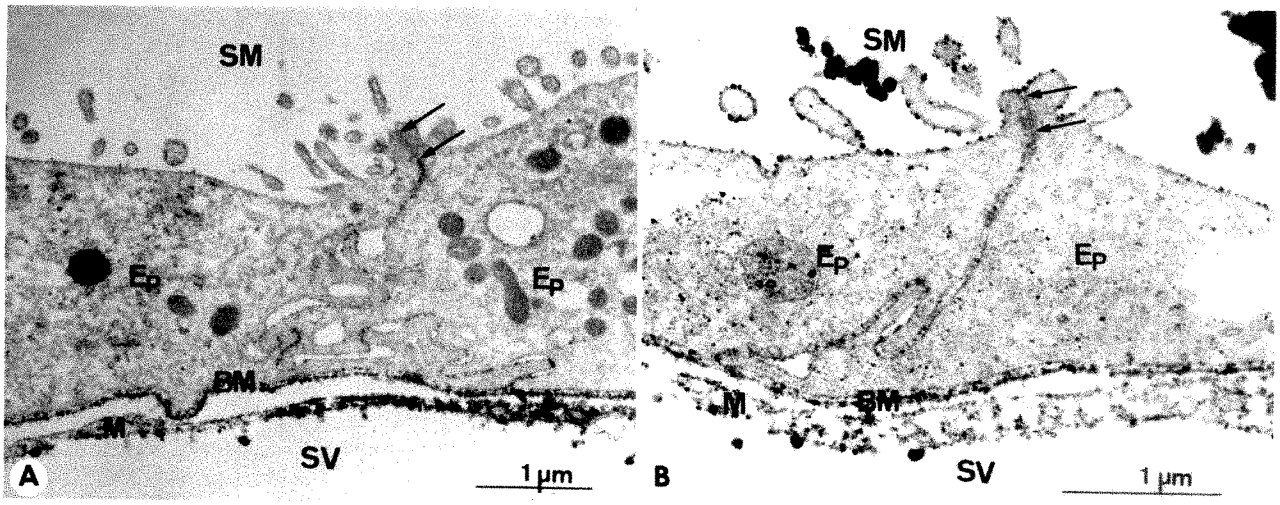

Fig 8. Reissner's membrane in horseradish peroxidase experiment. Second turn. M — mesothelial cell, BM — basement membrane, SV — scala vestibuli, SM — scala media, Ep — epithelial cells. A) Ten-minute perfusion. Mesothelial cell and BM on SV side are densely stained, but surface of SM is not stained. There is reaction product in intercellular space between Ep up to tight junctions (between arrows). B) Thirty-minute perfusion. Both endolymphatic and perilymphatic surfaces are stained. Tight junctions do not contain tracer (between arrows).

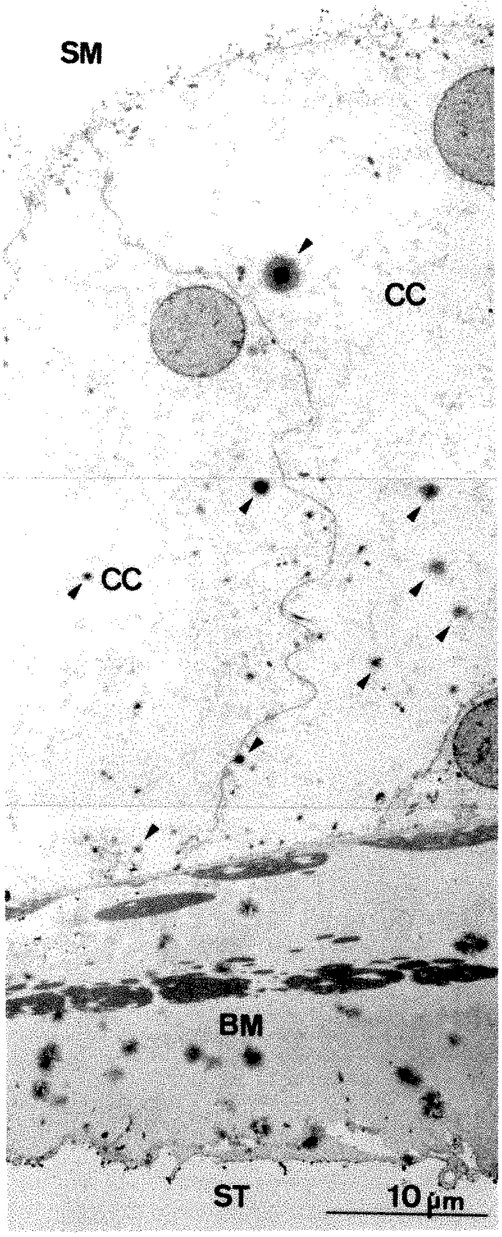

Fig 9. Thirty-minute perfusion of horseradish peroxidase, which attached to surface of Claudius' cell (CC) and mesothelial cells on scala tympani (ST). Number of fuzzy dots with core are observed throughout CCs (arrowheads) and basilar membrane (BM). SM — scala media.

present study indicated that two of five animals showed HRP in the scala media after a 30-minute perfusion (see Table). This suggested the possibility that macromolecules such as HRP may enter the scala media after a longer time.

From the findings of 5-OHDA and HRP as mentioned above, it appears that the transport of substances through the perilymph-endolymph barrier depends on the size of the tracer.

In general, the potential pathways for a small molecule from the perilymphatic space to the endolymphatic space are as follows: 1) an intercellular pathway through tight junctions and 2) a transcellular pathway by pinocytotic vesicles.

No labeling of 5-OHDA and HRP was observed in the tight junctions between the epithelial cells lining the scala media. Freeze-fracture studies have revealed that tight junctions are found between epithelial cell membranes in the stria vascularis,[6,20] in the reticular lamina of the organ of Corti,[21-23] in Hensen's cells and Claudius' cells,[6] and in Reissner's membrane.[6,24] Those studies suggested that the tight junctions formed an endolymphatic compartment apart from the perilymphatic space. When the freeze-fracture findings were compared to those of 5-OHDA, it was found that intercellular passage of a molecule as small as a neurotransmitter did not take place in the tight junctions lining the scala media.

Epithelial cells of Reissner's membrane showed labeled vesicles from the perilymphatic side to the endolymphatic side, as shown in Figs 2 and 8. This finding showed that 5-OHDA and HRP were taken up at the surface of the scala vestibuli, transported through the cytoplasm by vesicles, and released at the surface of the scala media, as reported in a ferritin experiment.[9]

There were many labeled pinocytotic vesicles in Claudius' cells and Hensen's cells in the 5-OHDA experiment. On the other hand, there were many fuzzy dots, which were considered to be reaction product of HRP, throughout the cytoplasm in the HRP experiment. Duvall and Quick[25] also reported that Claudius' cells were observed to phagocytize electron-dense tracers. Therefore, it is suggested that Claudius' cells and Hensen's cells provide a transcellular pathway for both tracers.

Surprisingly, the epithelial cells of the spiral prominence showed various degrees of degeneration after 30-minute perfusion of 5-OHDA, but not after either 10-minute perfusion or perfusion of artificial perilymph only. These observations could lead to the following hypothesis. The 5-OHDA was taken up selectively by diffusion and/or pinocytosis by the epithelial cells of the spiral prominence. Then it accumulated in the cytoplasm. The concentration of 5-OHDA eventually reached the level at which the tracer displayed a toxic effect. Finally, degeneration of epithelial cells occurred. This hypothesis supports the theory that the epithelial cells of the spiral prominence have an absorptive function.[9,26-28]

The reaction product of 5-OHDA filled the synaptic gap of outer hair cells, while it dotted other cell membranes (Fig 7). This indicated that 5-OHDA had a strong affinity to the synapse of the hair cell. However, cytochemical studies reported that no adrenergic fibers were found in the organ of Corti.[19,29] This discrepancy might be explained by the idea that there were adrenergic receptors in the synapse of hair cells and 5-OHDA bound to those receptors.

So far, the permeability of the perilymph-endolymph barrier has been discussed only from the viewpoint of molecular size. However, it is worthwhile to pay attention to electrical charge, solubility, and specific carrier system.[12] For better understanding of the transport of 5-OHDA and HRP, further study will be necessary.

In conclusion, the present findings suggest the following.

1. 5-Hydroxydopamine and HRP are transported from the perilymphatic space to the endolymphatic space. The degree of transport depends on the size of the particle.
2. The responsible sites for the passage are mainly Reissner's membrane, and possibly Claudius' cells and Hensen's cells.
3. The possible mechanism of the transport is pinocytosis.
4. Intercellular junctions between epithelial cells lining the scala media are still too tight for a molecule as small as a neurotransmitter.
5. The epithelial cells of the spiral prominence might have the function of absorbing endolymph.

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IMAGING CASE STUDY OF THE MONTH
MIDDLE EAR CHOLESTEROL GRANULOMA

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Middle ear masses are difficult to differentiate with computed tomography (CT) as the principal diagnostic tool. Occasionally the location or behavior of the lesion may help the differential diagnosis. Clinical evaluation has depended on observation of color through the tympanic membrane, to differentiate vascular (blue) and avascular (white) lesions. Magnetic resonance imaging (MRI) may be of further differential diagnostic use, as illustrated by the following example.

CASE REPORT

A 17-year-old girl presented with a history of progressive hearing loss on the right side. She had a

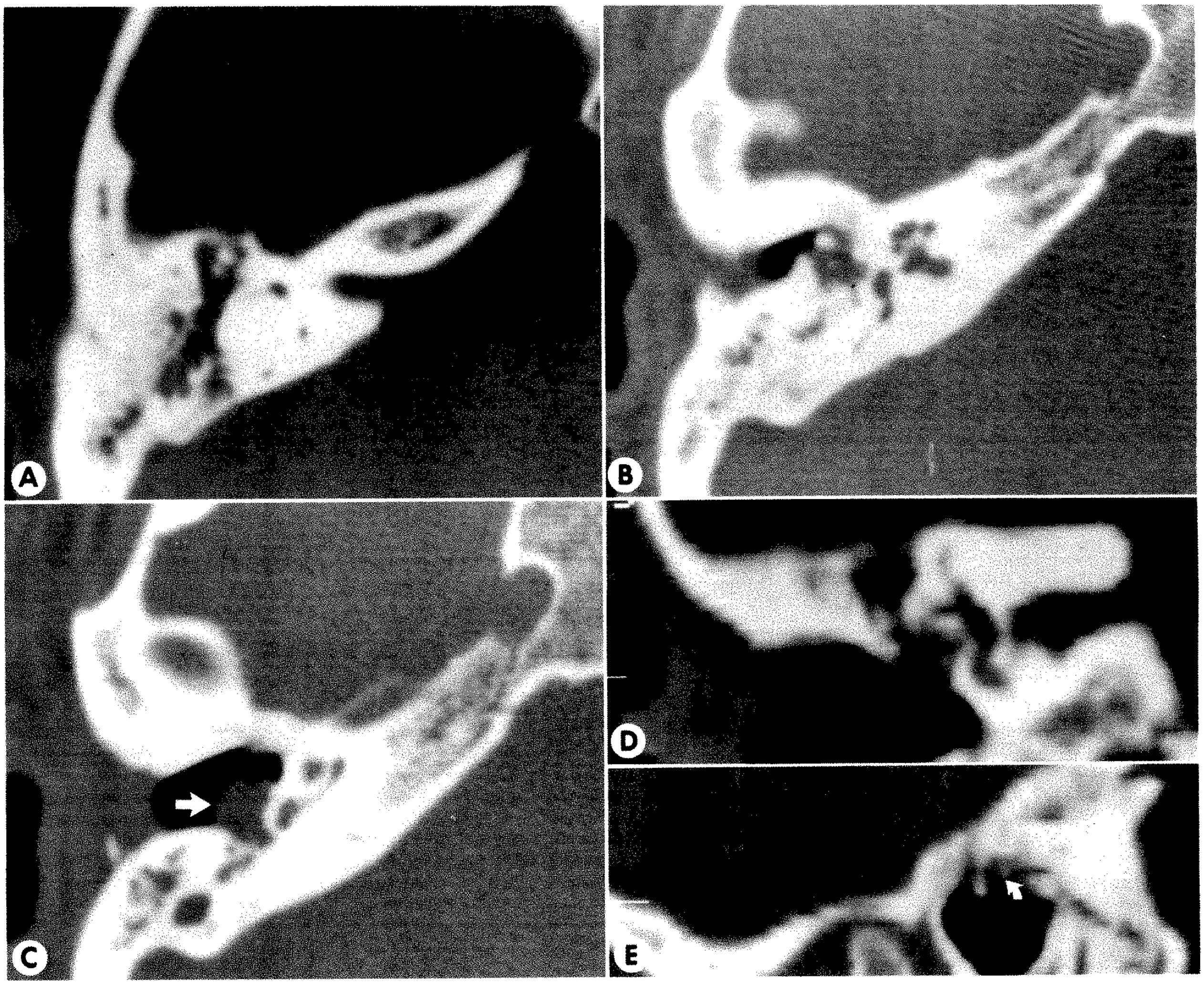

Fig 1. Computed tomograms. A) Axial view through attic and antrum showing opacification due to obstruction. No bone erosion present. B) Axial view 5 mm lower in area of sinus tympani. Mass fills mesotympanum. C) Axial view 3 mm lower in plane of basal turn. Oval mass fills sinus tympani posteriorly (arrow). D) Coronal reconstruction view through vestibule–oval window plane illustrating mass and intact scutum. E) Sagittal reconstruction view in plane of ossicles. Mass is evident. Erosion of long process of incus (arrow).

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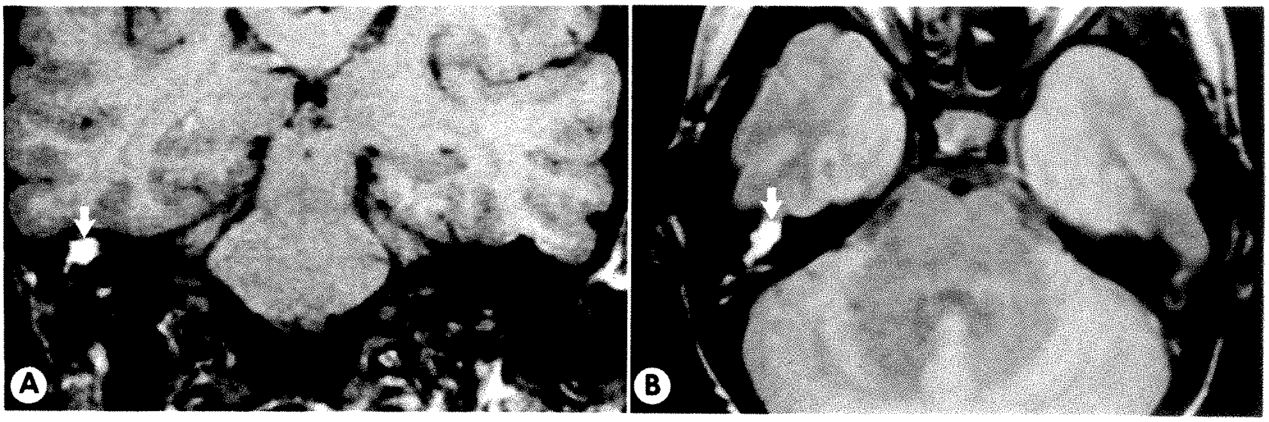

Fig 2. Magnetic resonance images with bright signal in granuloma (arrows). A) Coronal T1 view. B) Axial T2 view.

long history of an immunoglobulin (Ig) G immune deficiency complicated by many bouts of pneumonia and otitis media. She had a history of a left mastoidectomy for a cholesteatoma.

On clinical study the right ear had a posterior-superior blue mass behind the tympanic membrane. The mass was not pulsatile. The rest of the tympanic membrane was thick, white, and retracted against the promontory. There was no otorrhea. The patient had no vestibular symptoms. Audiometric examination revealed a 50-dB air-bone gap.

On CT examination the attic and antrum were opaque (Fig 1A). A mass became evident at the level of the oval window recess (Fig 1B). In this view the long process of the incus was located in the center of the mass and was barely visible. The stapes superstructure could not be identified, but the footplate was intact. The lowest view depicted (Fig 1C) shows the mass filling the sinus tympani. A coronal reconstruction view in the plane of the scutum and oval window recess (Fig 1D) shows the mass adjacent to the intact scutum. The long process of the incus and the lentiform stapes superstructure are not evident, but the footplate is intact. A sagittal reconstruction (Fig 1E) was made in the plane of the ossicles and shows the long process erosion as well as the oval mass.

Several diagnostic possibilities were suggested by the CT examination. Included were vascular lesions — most likely in view of the blue appearance of the mass as seen through the tympanic membrane.

T1 (Fig 2A) and T2 (Fig 2B) MRI examinations were performed. In each study, bright signal was obtained from the lesion. Only blood or cholesterin has this characteristic on MRI examination. Since the clinical examination excluded blood, cholesterin was suspected, and a cholesterol granuloma was thought to be the most likely cause of the lesion.

At operation a blue cystic mass was noted that yielded gelatinous brown-to-yellow fluid consistent with a cholesterol granuloma. The findings of the pathologic examination were consistent with a cholesterol granuloma.

DISCUSSION

A cholesterol granuloma represents a focal response to chronic middle ear infection. It consists of reactive foreign body giant cells that surround large cholesterol crystals. There is an associated mass of inflammatory cells, macrophages, and fibrous tissue. The cholesterol crystals are presumed to be the product of cellular degeneration secondary to chronic otitis media or other obstructing disease.[1] Grossly, the cholesterol granuloma is brown-to-yellow glistening tissue and may give the tympanic membrane a blue hue.

In contrast to the granuloma, a cholesteatoma represents a focal ingrowth of squamous epithelium associated with the production of keratin. The nidus of keratinizing epithelium is thought to originate from the external ear canal and then subsequently migrate to the middle ear through a defect in the tympanic membrane. Grossly, the cholesteatoma is a firm, white, pearly lesion. It contains keratinizing squamous epithelium, keratin, keratin debris, and fibrous tissue. The cholesteatoma usually produces very little signal on MRI examination.

Otoscopically, a blue tympanic membrane and associated mass may represent a cholesterol granuloma. The cholesterol granuloma may mimic a glomus tumor, high jugular bulb, aberrant internal carotid artery, hemotympanum, or tympanic extension of an intracranial meningioma.

Radiographically, cholesteatomas are associated with erosion or displacement of the ossicles, erosion of the lateral attic wall, facial nerve canal, and the superior wall of the external canal.[2] Cholesterol granulomas may also be associated with adjacent erosion of ossicles or bone.

The role of chronic otitis media in the development of cholesterol granulomas has been noted above. Additionally, IgG2 deficiency has been shown to be associated with a tendency toward recurrent otitis media. In otitis-prone children whose IgG2 levels are significantly deficient, otitis from *Hemophilus influenzae* infection occurs at rates as high as 2.6 times those in children with IgG2 levels above 0.60 g/L.[3]

The differential diagnosis of an expansile lesion of the petrous apex should also include primary bone and cartilaginous tumors, mucoceles, and neuromas.[4]

The CT appearance of cholesterol granulomas may be indistinguishable from that of cholesteatomas. The MRI appearance of the cholesterol granuloma is characteristically one of high signal intensity on both T1- and T2-weighted images because of the paramagnetic effects of protein content and cholesterin.[5,6] Cholesteatomas have been shown to be isointense or hypointense on T1-weighted images and of high signal intensity on T2-weighted images.

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SEVENTH INTERNATIONAL SYMPOSIUM ON THE FACIAL NERVE

The Seventh International Symposium on the Facial Nerve will take place June 9-14, 1992, in Cologne, Germany. For further information, please contact the Congress Secretary, Klinik und Poliklinik für HNO, Universität zu Köln, Kongressburo, Joseph-Stelzmann-Strasse 9, D-5000 Köln, Germany; 49-221-478-6190.

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MANAGEMENT OF CRANIAL BASE DISEASES

A one-day comprehensive review of contemporary management of cranial base diseases for neurosurgeons, otolaryngologists, ophthalmologists, radiation oncologists, and reconstructive surgeons will be held Nov 10, 1991, at the Ritz-Carlton Hotel in San Francisco, California. The course directors are Phillip H. Gutin, MD; Robert K. Jackler, MD; and Lawrence H. Pitts, MD. For further information, contact Extended Programs in Medical Education, Room LS-105, University of California School of Medicine, San Francisco, CA 94143-0742; (415) 476-4251.

Ann Otol Rhinol Laryngol 100:1991

PATHOLOGY CONSULTATION

SALIVARY GLAND TUMORS IN CHILDREN

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Fewer than 5% of all primary salivary gland neoplasms occur in children, but if benign supporting tissue tumors are excluded, a higher proportion than in adults are malignant. The first decade of life, and particularly the first 2 years of life, has a preponderance of benign neoplasms. Commencing with the second decade, carcinomas rise in incidence and are most often mucoepidermoid and acinic cell carcinomas. The pleomorphic adenoma is the most common epithelial salivary tumor throughout childhood. The embryoma may be a uniquely childhood epithelial salivary gland tumor.

No more than 5% of all salivary gland neoplasms occur in children. Table 1,[1-6] based on institutional data from six centers, indicates that 3.3% of the neoplasms, benign or malignant, present in patients who are 16 years of age or younger. Several features distinguish the neoplasms in this age group as compared to those in adults: 1) a much greater frequency of nonepithelial tumors, 2) a higher proportion of malignant neoplasms when nonepithelial tumors are excluded,[7] and 3) a preponderance of parotid gland involvement, eg, 7:1 ratio with submandibular gland.

Table 2[1,3,4,6,8-12] gives the histologic classification of 668 salivary gland tumors found in patients younger than 16 years. It should be noted, however, that there is a variation in frequency of histologic types according to the age of the child (Table 3[3,5,9,11,13,14]).

Vasoformative, nonepithelial tumors are the most common tumors in neonates and infants. The rarely occurring embryoma is almost confined to the perinatal-neonatal period. Pleomorphic adenoma, mucoepidermoid carcinoma, and acinic cell carcinoma occur later in childhood — nearly 80% between the ages of 10 and 16 years in the experience of Seifert et al.[7]

Parotid gland hemangiomas dominate the statistics for supporting tissue tumors.[4,11,15] They are the most frequent tumor of any type in the first year of life, and occur most often in girls and on the left side. A spontaneous involution over a few years' period is the usual course. Indications for surgical intervention include a persistence or sustained growth, extensive intratumoral hemorrhage, and an unsureness of the clinical diagnosis. Lymphangiomas are rare primary intraparotid vasoformative lesions. Usually the glands are incorporated by extraglandular lymphangiomatous extensions. Other reported benign, intraglandular, major salivary gland (usually parotid) tumors are neurofibromas, xanthomas, fibromatosis, and lipomas.[5,6,11,14,16]

Primary sarcomas of the salivary glands are seldom seen in children. Krolls et al[5] found 6 in their review of 168 neoplasms from children younger than 16 years; 5 were rhabdomyosarcomas. Eleven of the 18 primary salivary gland sarcomas reported by Auclair et al[17] were also rhabdomyosarcomas.

Of 3,875 pleomorphic adenomas accessioned at the Armed Forces Institute of Pathology, only 55 (1.4%) presented in pediatric (15 years or younger) patients.[5] Ten years is the age of peak incidence for the tumor, and it is the most common epithelial salivary gland tumor in children.[5,7,11] Monomorphic adenomas of any histologic subtype are very rare in patients younger than 16 years.[5,7,8]

Mucoepidermoid and acinic cell carcinomas, in that order, account for almost 60% of the malignant epithelial salivary neoplasms in children.[5,7,11] Other types, notably the adenoid cystic carcinoma, are rarely encountered.[18] The parotid gland is the site of predilection for all forms of salivary carcinoma in children. It should be of clinical interest that most of the mucoepidermoid carcinomas are diagnosed in patients in the 10- to 16-year age group, with the first 2 years of life relatively spared.[5,10,11,14]

TABLE 1. INCIDENCE OF SALIVARY GLAND TUMORS IN CHILDREN

Authors	*No. of Salivary Gland Tumors*	*No. in Children**
Bianchi and Cudmore[1]	937	12
Castro et al[2]	2,135	38
Catania et al[3]	657	11
Chong et al[4]	1,600	74
Krolls et al[5]	3,907	168
Schuller and McCabe[6]	587	19
Total	9,823	322 (3.3%)

*Patients under 16 years of age.

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TABLE 2. HISTOLOGIC TYPES OF SALIVARY GLAND TUMORS IN PATIENTS UNDER 16 YEARS OF AGE

Benign epithelial (50% of epithelial)	
Pleomorphic adenoma	182
Embryoma	5
Warthin's tumor	3
Cystadenoma	3
Lymphoepithelial lesion	3
Monomorphic adenoma	1
Malignant epithelial (50% of epithelial)	
Mucoepidermoid carcinoma	108
Acinic cell carcinoma	27
Adenocarcinoma	22
Undifferentiated carcinoma	15
Adenoid cystic carcinoma	11
Carcinoma ex pleomorphic adenoma	9
Squamous cell carcinoma	4
Unclassified carcinoma	1
Total epithelial	394 (58.9%)
Benign mesenchymal (93.8% of mesenchymal)	
Hemangioma	191
Lymphangioma	48
Neurogenic	11
Lipoma	4
Xanthoma	2
Fibromatosis	2
Malignant mesenchymal (6.2% of mesenchymal)	
Sarcoma	15
Ganglioneuroblastoma	1
Total mesenchymal	274 (41.0%)

Data from references 1, 3, 4, 6, and 8-12.

The majority of childhood mucoepidermoid carcinomas are histologically low grade, and they are seldom fatal.[5,7,9-11] Acinic cell carcinoma, the second most frequent carcinoma, manifests a similar age preference as the mucoepidermoid carcinoma and behaves like its counterpart in adults.

If there is an epithelial salivary gland tumor unique to children, the embryoma qualifies.[19] To date recognized only in newborns or during the first year of life, embryomas are morphologically similar to stages of development of the embryonic anlage of the major salivary glands. Less than 25% of the reported embryomas have shown histologic and/or biologic signs of malignancy. The majority have responded to surgical excision with a benign clinical course.[19]

A clear picture of the incidence of epithelial neoplasms of minor salivary glands in the pediatric age group is difficult to come by. The neoplasms, however, appear to be exclusively oral in location. The survey by Waldron et al[20] may be the most representative of the frequency in the general population. Of 424 patients with epithelial tumors of the intraoral minor salivary glands, 16 (3.8%) were younger than 20 years of age (only 1 younger than 9 years). Using the same upper age limit, Seifert et al[7] indicate that 17 of 80 (21%) salivary epithelial neoplasms arose from oral minor salivary glands.

There are only two histologic types — the pleomorphic adenoma and the mucoepidermoid carcinoma — recorded in compilations of intraoral salivary gland tumors in the pediatric group. Krolls et al,[5] who limited their case selection to patients 15 years or younger, and Waldron et al[20] present a 10:1 ratio of pleomorphic adenoma to mucoepidermoid carcinoma. Seifert et al[7] give a 2:1 ratio. In the Swedish Cancer Register there are no carcinomas of minor salivary glands in patients under 16 years either between 1958 and 1974 or in the decade ending in 1987.[21]

TABLE 3. DISTRIBUTION BY AGE OF SALIVARY GLAND TUMORS IN CHILDREN

Tumor Type	*Total No.*	*Perinatal*	*1-11 mo*	*1-10 y*	*10-16 y*	*Not Stated*
Benign epithelial						
Pleomorphic adenoma	81	1	1	27	52	
Embryoma	2	2				
Warthin's tumor	2					2
Cystadenoma	2					2
Basal cell adenoma	1	1				
Malignant epithelial						
Mucoepidermoid carcinoma	60		1	26	31	2
Acinic cell carcinoma	22			7	15	
Undifferentiated carcinoma	10	2	1	5	2	
Adenocarcinoma	8		1	4	3	
Adenoid cystic carcinoma	6			1	5	
Squamous cell carcinoma	4			1	3	
Benign mesenchymal						
Hemangioma	96	28	49	16	1	2
Lymphangioma	20			1		19
Neurogenic	7				1	6
Lipoma	2					2
Fibromatosis	3			3		
Malignant mesenchymal						
Sarcoma	18			2		16
Total	344	34	53	93	113	51

Data from references 3, 5, 9, 11, 13, and 14.

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FIRST INTERNATIONAL SKULL BASE CONGRESS

The First International Skull Base Congress will be held June 14-20, 1992, in Hanover, Germany. For further information, contact Madjid Samii, MD, Professor and Chairman of Neurosurgery, Medical School Hanover, Neurosurgical Clinic, Hospital Nordstadt, Haltenhoffstr 41, D-3000 Hanover 1, Germany; phone 0049-(0)511-7638245 or 623151, FAX 0049-(0)511-7638606.

LETTERS TO THE EDITOR

To the Editor:

We enjoyed reading the article by Moss et al (Cervical Necrotizing Fasciitis: An Uncommon Sequela to Dental Infection, *Ann Otol Rhinol Laryngol* 1990;99:643-6). Although the management of this devastating condition is basically well described, we wish to disagree with the statement "It is uncommon in necrotizing fasciitis to see muscle necrosis" (p 645). The term necrotizing fasciitis was coined by Wilson[1] because he considered the fascia to be the initial site of infection; however, the fulminating disease spreading mainly by thrombophlebitis rapidly afflicts the structures above and below the fascia. That is why radical debridement in this condition is well advised. In our experience with 15 cases of this entity,[2,3] we always found histologic features of myonecrosis in muscles adjacent to the infected fascia, even when the macroscopic appearance of this tissue was normal. Our conclusion, therefore, is that the disease is more widespread than its appearance indicates. Frozen section examination permitted us to detect contaminated tissues and to obtain disease-free surgical borders. The fewer the necrotic foci, the better the chances of cure. With such an approach, the mortality rates were lowered to 7%. The underlying debilitating condition, cited by Moss et al, did not adversely influence the outcome in our patients.

It is our impression that the authors did not clearly describe or emphasize some important points in the management of necrotizing infections. A Gram stain is efficient in recognizing the nature of the colonizing flora in these infections. It is of major importance in excluding clostridial gas gangrene, which necessitates a different antimicrobial regimen.

It seems appropriate to excise some of the skin overlying the infected area in order to keep the wound open (soft tissues exposed) rather than perform multiple skin incisions that may jeopardize the local vasculature and precipitate further ischemia. The open wound enables the early detection of new foci of infection and their prompt removal at repeated daily dressings. This may also avoid an undesirable, poorly aerated medium that favors the growth of anaerobic organisms.

In our experience skin grafts were not really effective in covering the denuded areas in the healing stages of these infections. They frequently become contaminated by *Pseudomonas* species. In small defects, the removal of excessive granulation tissue causes the encircling epithelium to rapidly bridge the gap. In large defects, local or regional flaps may better solve this problem by preventing the contractures that often occur in skin grafts.

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To the Editor:

According to some authors, necrotizing fasciitis (NF) is characterized by extensive necrosis of subcutaneous tissue and superficial fascia, with skin and muscle spared.[1-3] Gallia and Johnson[4] and some other authors[5-7] mentioned that it is a severe soft tissue infection characterized by extensive necrosis of superficial fascia, widespread undermining of surrounding tissue, and extreme systemic toxicity. Fisher et al[8] proposed six criteria of diagnosis of NF, including absence of muscle involvement. In the advance of the disease, all the surrounding soft tissue may be involved, including the muscles, bones, deep fascial spaces of the neck, mediastinum, and pericardial and pleural cavities.[6,7,9-19] Some authors prefer to term these conditions necrotizing soft tissue infection or necrotizing cellulitis,[6,7,12,13,15,16,19] but these terms still do not cover the necrosis of bone. Dellinger[15] described microscopic findings in this type of infection as consisting of focal necrosis with microabscess formation, and dense white blood cell infiltration in the subcutaneous tissue and superficial fascial plane, leaving the overlying skin and underlying muscle preserved, although thrombosis of arterioles and venules could be observed. Klabacha et al[16] classified severe soft tissue infection of the face and neck into four types. Type 3 NF has superficial fascia and its major plane infection, and the deeper infection of type 4 is in the muscle group.

The landmark for the disease-free surgical margin seems most definite when distinguished by frozen section, but practically it may take time in the operation and may worsen the outcome, especially in a severe case like ours. Generally, most surgeons demonstrate the line of demarcation of the uninvolved tissues by the normal colorization and active bleeding from the cut surface.[3,5,9] Stamenkovic and Lew[17] suggested the use of frozen section biopsy in early diagnosis of NF. Tehrani and Ledingham[20] found no histologic abnormality of full-thickness skin biopsies in the early stage. The subcutaneous fat and superficial fascia show a continuing nonspecific inflammatory reaction with fibrinoid arteriolitis and thrombosis of the vessels.

We congratulate Dr Tovi's group on the outcome of their NF treatment, which was not adversely influenced by the debilitating disease, as mentioned by others.[1-5,8,9,11,13,21]

A Gram stain is mandatory in every case of infection in which the fluid or specimen from the site of the lesion can be obtained. In NF, several authors recommended this as a primary guide for choice of antibiotics[1,8,11-13,19,21] and differentiation from clostridial infection.[8,15] Rouse et al[3] suggested the use of a Gram stain for the questionable area of involvement. Specific treatment should be started with intravenous penicillin and another broad-spectrum antibiotic that can cover gram-negative rods, even without waiting for the result of the Gram stain[1,8] or culture.[7,8,13,14] The rational selection of antiobitics can be made only with the knowledge of the microbiologic flora involved.[2,4,5,19] Our case report revealed gram-positive cocci and gram-negative rods, which we did not mention in our article.

Early recognition, prompt surgical debridement, and aggressive medical therapy are essential for treatment and reducing mortality of this entity.[1-4,6-9,11-21] The procedure includes resuscitation and stabilization of the patients, multiple linear incisions, and debridement. The need for one incision or more depends on the size of the area of involvement. The skin incision down to the necrotic area can expose the soft tissue adequately for drainage and oxygenation. Making an excision of the skin overlying the infected area seems necessary, provided the involved skin shows necrosis by appearing pale red, magenta, or purple, with blisters or bullae.[5,9,18] This leads to the healing process, which is usually by secondary intention but sometimes may need skin grafts,[1,3,5,9,11] a local flap, or a regional flap.[9] Multiple linear incisions may result in less damage to the vascularization of the surrounding tissues than would one incision with an extensive area of undermining.

I would like to thank Dr Tovi and his colleagues for sharing their experience and knowledge with me and bringing to my attention many problems of NF. There were a few more cases of NF in our department that were more efficiently treated. We performed both single and multiple incisions on them to obtain adequate drainage, and all are healed without a skin graft or local flap for repair.

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BOOK REVIEWS

Open Structure Rhinoplasty

Calvin M. Johnson, Jr, and Dean M. Toriumi. Hard cover, illustrated, indexed, 516 pages, 1990. WB Saunders, Philadelphia, Pa, $295.

Open Structure Rhinoplasty critically analyzes the authors' step-by-step approach to open rhinoplasty. The authors were motivated to write this text to clearly elucidate a method of rhinoplasty that permits the surgeon to operate on and modify the nasal structure under direct vision. In addition, they state that this technique allows observers and surgical assistants to readily appreciate the nasal anatomy and structural changes created by specific surgical steps.

The text is divided into 12 chapters that thoroughly deal with the basic concept of open rhinoplasty and aesthetic anatomy as it applies to each anatomic region of the external nasal pyramid. Chin augmentation and revision rhinoplasty are discussed following the basic surgical approach. In conclusion, numerous case studies are presented that detail the preoperative evaluation with color photographs highlighting major aesthetic problems. The corrective surgical steps employed are enumerated and potential problem areas discussed.

Each chapter contains numerous appropriate illustrations as well as preoperative and postoperative color photographs. The case study chapter is particularly well illustrated and contains numerous color photographs depicting preoperative and postoperative results dealing with primary and revision rhinoplasty cases.

This is a practical textbook that is easy to read and holds one's interest from cover to cover. Since the information presented ranges from basic tenets to detailed revision surgery, it would definitely make a valuable addition to any library. It presents an anatomic and clinical discussion of an approach to rhinoplasty that has gained a substantial following and is certainly appropriate in specific cases.

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New York, New York

Clinical Neurophysiology of the Vestibular System

Robert W. Baloh and Vicente Honrubia. 2nd ed. Volume 32 in *Contemporary Neurology Series.* Hard cover, illustrated, indexed, 301 pages, 1990. FA Davis, Philadelphia, Pa, $65.

This volume (number 32) of the *Contemporary Neurology Series* represents a timely second edition of the original text published in 1979. Its purpose as stated by the authors is "to provide a framework for understanding the pathophysiology of diseases involving the vestibular system." The authors accomplish this purpose as well as is currently possible, drawing on the dual perspectives of the authors (the first, primarily a neurologist, the second, an otolaryngologist).

The text is logically divided into three parts. The first, and clearly the finest, reflects the basic science interests of the authors in clinical neuroanatomy and physiology. Detailed mathematics of cupular dynamics and visual-vestibular interaction append this section at the end of the volume. This section has been studded with phylogeny in order to illustrate its points. The authors draw their extensive bibliography from many disparate scientific and clinical disciplines, further increasing the breadth of perspective.

The second section, entitled "Evaluation of the Dizzy Patient," is less satisfying. This is the fault not primarily of the authors, but of the current vexing state of affairs in the evaluation of patients with balance disorders. The chapter on history-taking is fairly complete, with concise compilation of material available from many sources. The authors similarly provide a succinct account of "bedside" physical examination, noting in most cases that responses are "nonspecific" or "difficult to interpret." This theme continues into the chapters dealing with laboratory vestibular evaluation. The chapter on electronystagmography is illustrative and currently adequate. The chapter on rotational tests again reflects the authors' special interest, including again the oft-reported tests of visual-vestibular interaction. Newer tests (eg, eccentric rotation, track acceleration, and moving-platform posturography) are mentioned superficially. We found notable the multiplicity of imprecise tests of vestibular evaluation enumerated in this section with the exclusion of contemporary radiology, our most sharply defining modality. Vestibular evaluation has apparently reached the stage of audiology prior to the appearance of brain stem evoked response audiometry. This parallel is made poignant by the final chapter of the section, "Clinical Evaluation of Hearing."

The third section is entitled "Diagnosis and Management of Common Neurotologic Disorders." This section departs from the original premise of the authors, in that it seems to be a comprehensive "handbook" of disorders rather than a treatise on pathophysiology. Certainly, the constraints of space as well as the desirability of clinical relevance mandate this departure. Nonetheless, the enumeration of pathologic processes in this section is impressive and clinically useful.

In essence, this text attempts to simultaneously satisfy two related and overlapping audiences. The first part of the volume delves into its topic for the serious students and researchers of vestibular physiology. The last attempts to avail the clinician of a vast body of pathology in an easily accessible and concise format. We found this dichotomy interesting and overall commendable.

JEFFREY B. BANYAS, MD
DENNIS G. PAPPAS, MD
Birmingham, Alabama

Management of Blunt Trauma

Robert Y. McMurtry and Barry A. McClellan, editors. Hard cover, illustrated, indexed, 479 pages, 1990. Williams & Wilkins, Baltimore, Md, $99.

This volume addresses a need for a generalized view of trauma management. Forty-three authors from 15 medical disciplines have each contributed according to their special expertise. Space, of course, assumes a high priority in this generalized, multispecialty text.

The reader can expect concise treatment of various topics germane to trauma management without slashing through the underbrush of controversy. The reader motivated by one of the smaller topics, however, will find little in this volume to stimulate him. The chapter on facial trauma, authored by an internationally recognized expert, is as complete in scope as one could expect in 31 pages. The chapter, nonetheless, would fail to satisfy the needs of a surgeon dealing primarily with facial trauma because of lack of depth.

In summary, then, this text is geared more to general surgeons and those primarily responsible for the administration of the trauma unit as a whole than to otolaryngologists.

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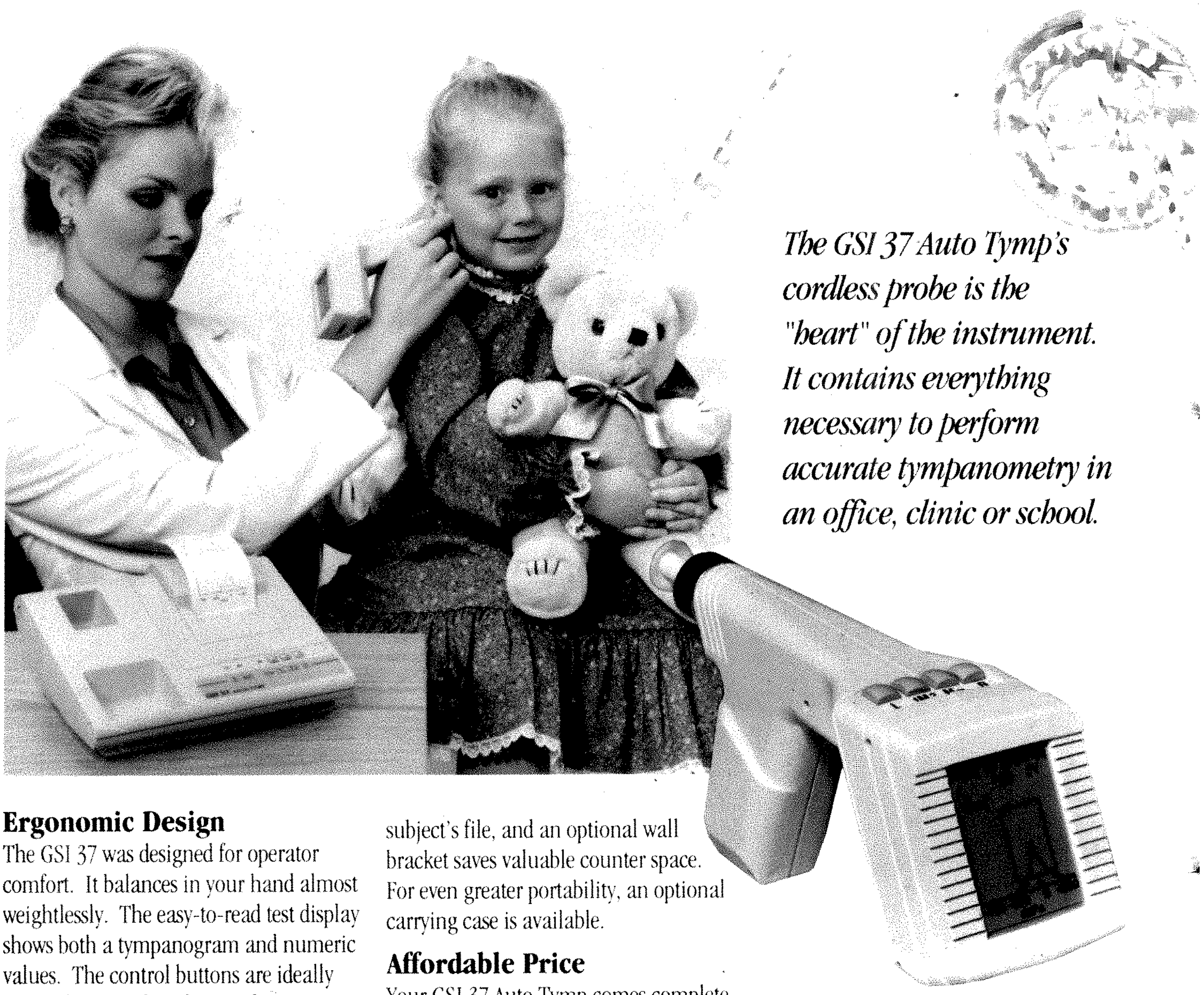

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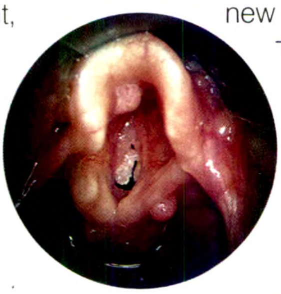

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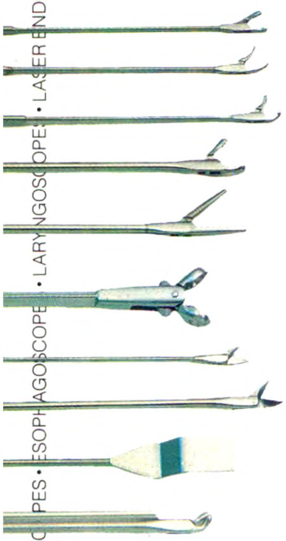

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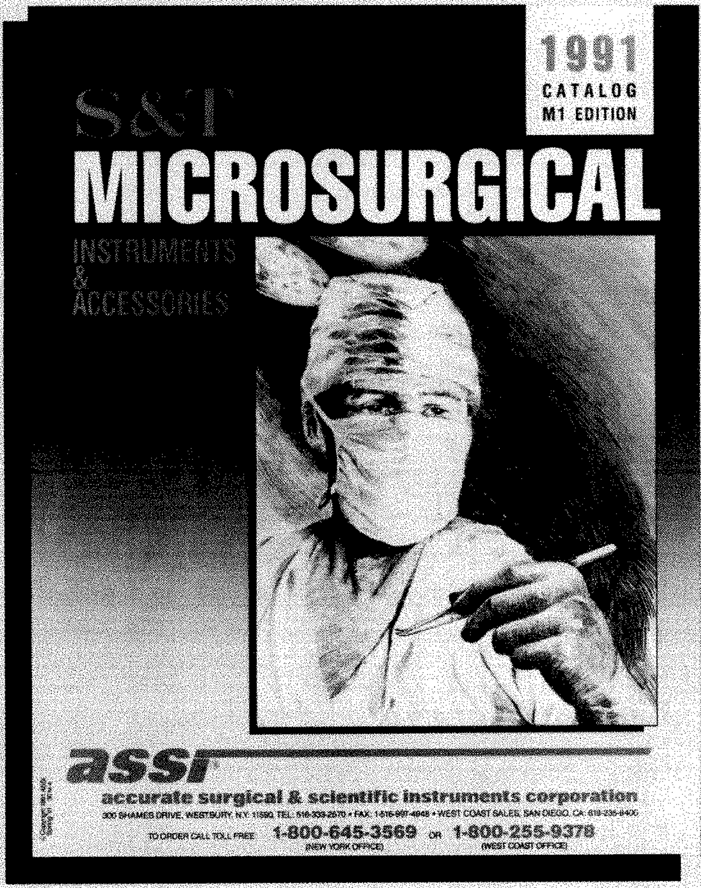
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EDITORIAL

MOVING INTO THE 21ST CENTURY: COMPUTER APPLICATIONS AND SOFTWARE REVIEW DEPARTMENT IN THE *ANNALS*

Over the past two decades our society has become increasingly dependent upon computers. I remember in the 1970s when it was predicted that soon one of every four homes in the United States would own a calculator. At the time, I was naive enough to doubt the statistic. Today calculators are ubiquitous, even distributed as promotional items by supermarkets and drug companies. Computers soon followed and are infiltrating every aspect of life. Presently 25% of all American homes have a personal computer and 2 million households have access to electronic bulletin boards (personal communication, A. Bose, Link Resources, 1991).

Medicine has become increasingly dependent upon computers also. Currently, 40% of medical and health-related small businesses are computerized, with an average of two personal computers for every 10 employees (personal communication, A. Bose). Forty-four percent of *all* otolaryngologists have a personal computer in their home and/or office (personal communication, Business Mailers, Inc, 1991). Tasks such as office billing, appointment scheduling, and literature searching can now be automated and laboratory and radiologic test results can be immediately retrieved. Computers have directly improved our ability to make diagnoses and treat patients. The first truly revolutionary application of computer science to health care was the computerized tomographic scan. Other uses loom on the horizon that will, without much doubt, apply artificial intelligence in the formulation of a differential diagnosis and even in the application of image-directed robotics in surgical procedures.

Computer programming and software development are viewed currently by most postgraduate institutions as an academic activity and are awarded academic credit toward advancement. However, despite this, only a few peer-reviewed journals are currently informing their readers about new software. The most prominent examples are *JAMA* and *Science*. In response to this rapidly growing and changing field, the *ANNALS* is establishing a computer software review section. This section will feature reviews of software submitted by authors and manuscripts describing computer applications in otolaryngology–head and neck surgery.

I am pleased to announce the appointment of Kevin T. Kavanagh, MS, MD, as the first editor of this new department. The guidelines for submission and review of manuscripts and software follow.

Brian F. McCabe, MD
Editor

GUIDELINES FOR SUBMITTING SOFTWARE AND MANUSCRIPTS ON COMPUTER APPLICATIONS TO THE *ANNALS*

I. *Software.* Programs must be compatible with an IBM or Macintosh computer and the author must specify hardware and system requirements. The following operating systems will be supported: MS-DOS; MS-DOS with Windows; or MAC-OS. The software may be submitted on either a 3.5" or 5.25" disk. If the program is a template, a runtime version of the source program or template must also be submitted. The reviewer may keep and use the software, regardless of whether or not the review is published. The software author is not obligated to provide updates or user support to the reviewer, although the reviewer may contact the software author with questions regarding software operation. As with book reviews, the reviewer's name will be published with the review. If the software author does not agree with the review, he or she may respond in a letter to the editor.

Software that will be considered for review includes the following:

1. Educational software, eg, instructional programs on inner ear anatomy.
2. Artificial intelligence software to aid in the diagnosis of otolaryngic disorders, eg, a program that will predict the etiology of vertigo based upon a set of patient symptoms and laboratory test results.
3. Software for logging operations performed by individuals, residents, and otolaryngology–head and neck surgery departments.
4. Software to aid in research, eg, data base and data management systems.
5. Software that addresses specific problems in otolaryngology.

II. *Manuscripts Reviewing Computer Applications Designed for Accomplishing a Specific Task.* The focus of this type of manuscript would be a review designed to advise the reader on how to purchase and evaluate software for accomplishing specific tasks. The article must relate directly to otolaryngology. For example, a comparison of the various online literature searching services should include a description of performance using various otorhinolaryngological subject headings.

III. *Manuscripts With a Problem/Solution Format.* This type of manuscript should be written by the author of the software or someone who has used it extensively. The authors should describe a specific problem, explain how the software solves the problem, and provide data verifying the effectiveness of the solution. In order to meet these criteria the manuscript must have two characteristics. First, the computer software should be unique, and not a duplicate of other products capable of performing the same or similar tasks. Second, the effectiveness of the software should be demonstrated objectively with data. If no data are present, then the judgment of effectiveness is subjective and the software itself should be submitted for review in lieu of a manuscript.

Submitted manuscripts will be peer-reviewed by at least two reviewers who are experts in the field. All reviews, unidentified as to reviewer, will be returned to the submitting author. Our goal for this new section in the *ANNALS* is to keep pace with and further stimulate the rapid development of medical informatics and computer science in otolaryngology–head and neck surgery.

Submit materials to Brian F. McCabe, MD, Editor, Department of Otolaryngology–Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

Kevin T. Kavanagh, MS, MD
Software Review and Computer
Applications Editor

Ann Otol Rhinol Laryngol 100:1991

PSYCHOLOGICAL CHANGE FOLLOWING 18 MONTHS OF COCHLEAR IMPLANT USE

JOHN F. KNUTSON, PHD HELEN A. SCHARTZ, MA BRUCE J. GANTZ, MD
RICHARD S. TYLER, PHD JAMES V. HINRICHS, PHD GEORGE WOODWORTH, PHD
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Consecutive recipients of multichannel cochlear implants participated in preimplant as well as 9-month and 18-month psychological evaluations. Before receiving a cochlear implant, psychological tests indicated that the implant recipients were more depressed, suspicious, socially isolated, lonely, and socially anxious than was the general population. After 18 months of implant use, there was a significant reduction in depression, loneliness, social anxiety, social isolation, and suspiciousness. These changes in psychological state did not correlate with improved performance on audiological measures. The data suggest that although cochlear implants can have a positive effect on the emotional and behavioral status of persons with acquired postlingual profound deafness, the psychological outcome of implants is not simply a function of the audiological benefit assessed with standardized speech-based audiological tests.

KEY WORDS — audiological tests, cochlear implant, psychological change.

Although there is a paucity of data regarding psychological consequences of an acquired profound hearing loss, existing evidence based on persons with severe hearing loss suggests that acquired deafness is likely to be associated with significant disruptions in many areas of psychological functioning.[1-4] On the basis of an assessment of large cohorts of subjects, Thomas[4] provided evidence that persons with acquired hearing loss had prevalence rates of psychological disturbance approximately four times that of the general population. Problems in psychological functioning were typically manifested in interpersonal distress, affective disturbance, and loneliness. While there is apparent consensus regarding risk for these psychological problems among persons with acquired hearing loss, risk for other psychological difficulties in samples of subjects with acquired hearing loss is more controversial. For example, Thomas[4] and others[5] have strongly criticized the commonly held notion that hearing loss is associated with increased suspiciousness. Yet existing data obtained from samples of subjects presenting with a pure tone average hearing loss in excess of 95 dB hearing level (HL)[6,7] suggest that persons with profound hearing loss can display significant suspiciousness on psychological tests. Regardless of the specific characteristics of the psychological dysfunction, the presence of psychological distress is one factor influencing the decision by many postlingually deaf persons to seek a cochlear implant.

Although there is evidence that the current generation of multichannel cochlear implants can result in significant audiological improvement,[8-10] there is considerable variability in the audiological outcome of cochlear implants. Because of the variability in audiological outcome, other indices of change should also be considered in evaluating implant success. The psychological distress associated with profound hearing loss suggests that psychological outcomes of implants could provide a logical addition to the usual audiological measures. The possibility of using psychological indices of outcome would not be unique to cochlear implants; it has been argued that behavior and quality of life are central to evaluating the effectiveness of many medical procedures.[11]

The present study was designed to assess changes in psychological status in multichannel cochlear implant recipients who could be considered representative of the population of implant recipients and who had extensive experience with the device. Because audiological improvement following the introduction of a cochlear implant shows a rather gradual trajectory, it is important that indices of psychological change be monitored after the recipient has had sufficient experience with the device to achieve optimal audiological benefit. Data from this laboratory suggest that at least an 18-month follow-up period may be required. The areas of psychological functioning that were the focus of the present research included those areas about which there is considerable agreement regarding psychological disturbance in postlingually deaf samples (eg, depression, social isolation, loneliness, social anxiety) as well as an area about which there is considerable controversy (suspiciousness). The latter was included because of evidence that implant candidates evidence relatively high levels of suspiciousness relative to the general population.[6,7] Thus, the

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From the Departments of Psychology (Knutson, Schartz, Hinrichs), Otolaryngology–Head and Neck Surgery (Gantz, Tyler), and Statistics (Woodworth), The University of Iowa, Iowa City, Iowa. Supported in part by National Institutes of Health (NIH) grant DC 00242; grant RR59 from the General Clinical Research Centers Program–Division of Research Resources, NIH; and a grant from the Lions Clubs of Iowa.

Presented at the Second International Cochlear Implant Symposium, Iowa City, Iowa, June 4-8, 1990.

REPRINTS — John F. Knutson, PhD, Dept of Psychology, The University of Iowa, Iowa City, IA 52242.

present research was designed to determine whether psychological problems associated with acquired profound hearing loss would be ameliorated by extended cochlear implant use.

METHOD

Subjects. Subjects were 30 consecutively referred multichannel cochlear implant recipients who received their implant as part of the Iowa Cochlear Implant Project and who had completed 18 months of implant use. To be eligible for a cochlear implant in the Iowa program, subjects were required to evidence a profound (pure tone average >95 dB HL) bilateral, postlingually acquired hearing loss. Subjects ranged in age from 28 to 71 (mean = 52.5, SD = 13.8). None scored higher than 4% correct when NU-6 Monosyllabic Word Test lists were presented at 60 dB HL in the sound field with the subject wearing an appropriately fitted hearing aid. Under aided conditions, none understood more than 10% of the words from the auditory-only presentation of the Sentence Test Without Context–Sound Only task from the Iowa Phoneme and Sentence Test.[12]

Because the Iowa cochlear implant research protocol entails regularly scheduled follow-up sessions for 5 years, and because many of the test procedures require research participants to be literate and to be able to respond to a broad range of test materials, several psychological factors were included among criteria used to select research participants. Namely, implant candidates could not present with a severe psychiatric illness (eg, schizophrenia) or a severe personality disorder that would reduce the likelihood of participating in the follow-up protocol. Because of the intellectual demands of the research protocol, subjects were also required to evidence measured intelligence (Wechsler Adult Intelligence Scale–Revised) in or above the low-average range (IQ > 74). These psychological selection criteria were predicated on the demands of the research protocol and not on their possible relevance to implant outcome.

Although the criteria for admission to the research protocol truncate the range of IQ scores at the lower end of the distribution, the obtained sample evidenced a broad range of intellectual abilities. Only subjects more than 1.6 SD below the general population mean were actually excluded from the sample.

Procedure. Candidates presenting for a cochlear implant in this program participate in extensive medical, radiological, audiological, and psychological assessments distributed over 4 days. This preimplant evaluation is completed no more than 3 months prior to operation. Subjects who were to receive a cochlear implant in the program were assigned to receive either the 21-channel pulsed-signal Nucleus implant[13] or the 4-channel Ineraid analog-signal device,[14] on an alternating assignment schedule.

The preimplant psychological evaluation was typically scheduled after subjects had met the audiological, medical, and radiological criteria for implant eligibility. Thus, the psychological evaluation was the final stage of the preimplant assessment procedure. The psychological evaluation entailed a full day of interviews, questionnaires, and standardized psychological tests. Although it has been suggested that standardized psychological measures are inadequate for the task of evaluating implant candidates or assessing psychological variables relevant to implant success,[15] standardized instruments have established reliability and validity as well as enhanced prospects for replication among laboratories. It is important to note we included in this study only the postlingually deaf, so there was no language problem in testing. The psychological assessments included variables suitable for screening subjects for the research protocol such as measures of intellectual functioning and diagnostic interviews, as well as a series of tests of cognitive function that were being investigated as possible predictors of audiological outcome. The psychological variables that are the focus of the present study included measures of affect, social distress, and interpersonal difficulties.

Psychological Measures. The full Minnesota Multiphasic Personality Inventory (MMPI)[16] was administered, but only the Depression (scale 2), Paranoia (scale 6), and Social Introversion (scale 0) scales were included for analysis. As a second, but not redundant, measure of depressed affect, the Beck Depression Inventory[17] was administered. The Beck Depression Inventory was included because it is more sensitive to transient mood shifts than is the Depression scale of the MMPI. To objectively assess interpersonal distress that is experienced in social situations or is due to impaired social interaction, several measures were included. The UCLA Loneliness Scale[18,19] was included because of the expressions of loneliness often reported by implant candidates. Since loneliness may be due either to limited opportunities for interaction or to active withdrawal from social situations, a measure of anxiety and distress experienced in social situations was included: the Social Anxiety and Distress Scale.[20]

Audiological Measures. The audiological outcome measures were selected to assess several capabilities to respond to acoustic events that are functionally related to speech signals. All of the audiological tests were administered in a sound-attenuated chamber with use of a computer-controlled laser videodisc system at a level of about 65 dB sound pressure level. The following tests were administered in a sound-only (without visual cues) format: the Consonants Confusion Test (Consonants) and the Vowel Test (Vowel),[12] Phoneme

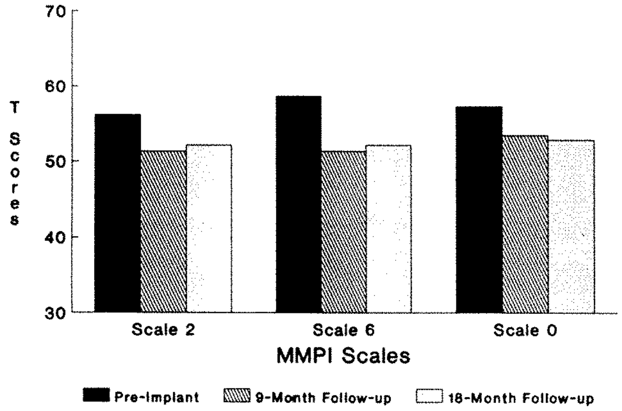

Fig 1. Mean Minnesota Multiphasic Personality Inventory (MMPI) scale scores at each test session. Scale 2 is Depression, scale 6 is Paranoia, and scale 0 is Social Introversion. Scale scores have population mean of 50 and SD of 10, with more clinically deviant scores in elevated direction.

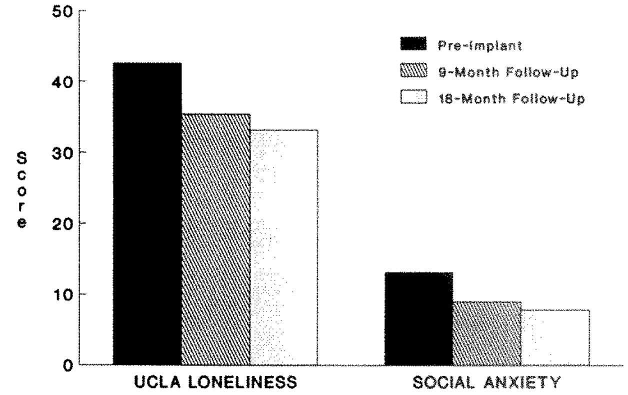

Fig 2. Mean UCLA Loneliness Scale scores and Social Anxiety and Distress Scale scores at each test session. Higher scores reflect greater difficulty on both scales.

Identification from the NU-6 word list (NU-6), and the Sentence Test Without Context-Sound Only task (Sentences) from the Iowa Phoneme and Sentence Test.[12] The Consonants and the Sentences tests were also administered in a sound-plus-vision format using a video monitor and the videodisc system to assess the impact of visual information.

RESULTS

A preliminary comparison of the audiological outcomes of the two implant types indicated that there were no statistically significant differences between devices (all $Fs<2.0$). Therefore, the data were aggregated across implant type for all analyses. Figure 1 shows the preimplant and follow-up mean scores for each of the MMPI scales considered. Relative to general population norms, this sample presented with a slight elevation on scale 2 (Depression) and elevations on scales 0 (Social Introversion) and 6 (Paranoia) that approached 1 SD above the general population mean. Changes in these MMPI scores across the follow-up sessions during the first 18 months of implant use were assessed with within-subjects analyses of variance. As reflected in the change in scale 2, there was a statistically significant decline in depressed affect across sessions ($F_{2,58}=4.41$, $p<.025$). Similarly, a statistically significant decline in suspiciousness was reflected in the change in scale 6 scores between the preimplant assessment and the follow-up sessions scheduled at 9 and 18 months postoperation ($F_{2,58}=11.99$, $p<.001$). The obvious decline in social introversion (scale 0) across assessment sessions was also statistically significant ($F_{2,58}=6.27$, $p<.005$). Thus, the mean of all three MMPI scales that were the focus of this research evidenced a significant decline to a level approximating that of the general population mean.

The UCLA Loneliness Scale assesses a perception of feeling lonely that is related to but not redundant with scale 0 of the MMPI. The UCLA scores obtained before implantation and at the two follow-up sessions are shown in Fig 2. At the preoperative session, the implant recipients reported extreme loneliness. The statistically significant decline in UCLA scores across test sessions shown in Fig 2 ($F_{2,58}=18.58$, $p<.001$) indicates that the implant recipients reported marked reductions in loneliness over the course of 18 months of implant use. These UCLA scores, however, suggest that the sample continues to evidence considerable loneliness after 18 months of implant experience. The preimplant and follow-up Social Anxiety and Distress Scale scores are also shown in Fig 2. Consistent with the decline in loneliness was a statistically significant ($F_{2,58}=10.61$, $p<.001$) decline in the experience of anxiety and distress in social settings across 18 months of implant use.

In comparing the two measures of depression, the Beck Depression Inventory is more a measure of depressive symptomatology than is the MMPI scale 2, and for that reason is not duplicative of the MMPI scale 2. The mean Beck Depression Inventory score prior to receipt of an implant fell in the mild to nondepressed range. Although there was a decline in the mean Beck Depression Inventory score to 4.6 after 18 months of implant use, this decline did not approach statistical significance. Thus, of the five measures of psychological change considered in this research, only the Beck Depression Inventory failed to evidence a statistically significant decline after 18 months of implant use. Since this measure was not significantly elevated at the preimplant session, a decline would be difficult to detect.

The changes in psychological functioning that occurred following the introduction of the multichannel cochlear implants suggest that audiological gains that result from the implant are associated with improvements in psychological functioning. To determine whether the degree of psychological change is a function of the degree of audiological improvement, the changes from preimplant to 18-month follow-up on the psychological measures were correlated with improvement on the six audiological outcome measures obtained at the 18-month follow-up session. Of the 18 correlations involving MMPI change scores, only the correlation between change on MMPI scale 0 and the Consonants test administered in a sound-plus-vision format was statistically significant ($r = .32$, $p < .05$). None of the correlations between the audiological measures and the UCLA Loneliness Scale change scores were statistically significant. The correlation between the change scores on the Social Anxiety and Distress Scale test and the sound-only Consonants test was statistically significant ($r = .43$, $p < .01$). The Consonants and the Sentences tests administered in the sound-plus-vision format were also significantly correlated with the Social Anxiety and Distress change score ($r = .38$ and $r = .37$, respectively). Thus, among the psychological variables that changed significantly from preimplant to 18-month follow-up, the degree of change was generally not predicted from audiological change. Indeed, only the Social Anxiety and Distress score change correlated with audiological improvement at a level beyond what could be expected to occur by chance.

Because the magnitude of change in psychological variables was not significantly related to the selected measures of audiological performance, an alternative approach to relating psychological improvement to improved audiological ability was considered. Because most of the psychological functioning assessed in the present study reflects social interaction, improved ability to lip-read was selected as an alternative to the original audiological measures for predicting psychological change. In these post-hoc analyses three different measures of evaluating lipreading improvement were used, with performance on the Sentences test providing the basic data. The first measure of lipreading enhancement was the difference between the Sound-Plus-Vision Sentences Test at 18 months and the Vision-Only test at the preimplant assessment. The second approach was the Sound-Plus-Vision Sentences score at 18 months divided by the preimplant Vision-Only Sentences score. The third, a measure of relative change, was the difference between the sound-plus-vision and vision-only scores divided by 100 minus the vision-only score. Although derived from the same data, these three approaches provide rather different metrics regarding degree of improvement in lipreading realized by implant recipients. Nevertheless, regardless of their different metric properties, there were no differences among the correlations between lipreading enhancement and psychological change. Only two of the correlations between lipreading enhancement and psychological change approached statistical significance.

DISCUSSION

The results of the present study indicate that the current generation of multichannel cochlear implants can result in reports of significant improvement in psychological functioning in persons with acquired profound hearing loss. The reported psychological improvements are consistent with observations reported by Tyler and Kelsay[21] in which better implant recipients reported areas of life change. The present study obtained evidence of improvement with objective measures having appropriate psychometric properties, and the changes were systematically evidenced across an 18-month period. Moreover, the areas of improvement involved depression, social isolation, and loneliness — areas that are within domains of psychological dysfunction among persons with acquired hearing loss.[3,4] The change in suspiciousness is not inconsistent with some evidence regarding psychological problems among persons with an acquired profound hearing loss.[6,7] Thus, the theoretical consistency of the data and the systematic nature of the obtained changes strongly support the hypothesis that multichannel cochlear implants can result in improved behavioral and emotional function in postlingually deafened adults. Conclusions regarding the functional relationship between the cochlear implant and the psychological outcome may be compromised, however, by the overall failure to relate degree of psychological change to degree of audiological improvement.

For all audiological measures used, there was considerable variability in performance. Similarly, there was variability in the psychological change measures. Thus, the failure to obtain significant correlations between audiological outcomes and psychological change cannot be attributed to restricted range, and statistical properties of the data cannot be invoked to account for the failure to correlate psychological change with audiological change. In view of the lack of a relationship between audiological benefit and psychological change, it might be argued that these psychological changes are epiphenomenal or that they merely reflect some nonspecific therapeutic influence analogous to a placebo effect. The systematic nature of the changes and the fact that the measures used often show considerable stability over time suggest that those possibilities are not likely. Nevertheless, in the absence of suitable comparison groups, these possibilities cannot be dismissed. Since waiting list controls are unacceptable, since there is no comparable alternative treatment acceptable to implant candidates, and since subjects denied an implant for medical reasons would not yield appropriate control groups, investigators attempting to address possible accounts of the psychological effects are confronted with serious control group limitations. Indeed, that is why there are no control groups in the present research protocol.

There are several alternative hypotheses that may be advanced to account for the obtained results. First, it is possible that the audiological measures that were used in the present analyses are simply not suitable for predicting psychological change. There is no single benchmark for assessing the audiological outcomes of implants. Although it seems likely that ability to understand speech should be critically important for psychological change, many successful implant recipients do not achieve a level of performance that approaches "normal hearing." Given limited ability to understand speech, but given the realization of some audiological benefit, it is possible that other indices of audiological improvement may be more related to psychological benefit than are the speech-related measures. Interviews with implant recipients, who demonstrate only modest benefit on audiological tests, suggest there could be psychologically important audiological benefits that are realized but perhaps not tapped by the usual audiological measures. The challenge confronting implant researchers may be to identify audiological measures that are psychologically important.

It is also possible that the obtained psychological change reflects a threshold function, namely that any audiological benefit will result in some psychological change. It may be reasonable to assume that any audiological benefit is an improvement and that as many implant recipients note, "leaving the world of silence" is psychologically important. The data of the present study, however, do not support such a simple threshold model. Although all subjects evidenced some audiological benefit, not all subjects evidenced psychological improvement. A more complex threshold model would be consistent with the obtained data. In such a model, audiological benefit is necessary but not sufficient for psychological change to occur. Since all of these implant recipients achieved some audiological benefit from the cochlear implant, it is possible that other factors operate to determine whether psychological benefit is experienced. During an 18-month follow-up there are many events that impinge on implant recipients that could have had psychological effects. For example, implant recipients experienced the death of a spouse, a divorce, and a newly diagnosed chronic illness, as well as new employment and social opportunities. Perhaps it is too much to expect that audiological improvement alone could account for the degree of psychological change following receipt of an implant. Audiological benefit from an implant, however, may make it possible for other life events to have a psychological impact.

It is also possible that the impact of audiological benefit may be a function of the expectations that the implant recipient brings to the procedure. While the research project in which these subjects participated provides full informed consent, it must be recognized that all participants have some hope for achieving maximum benefits, and that the outcome actually experienced reflects only an approximation of that hope. Perhaps psychological benefits reflect the degree to which personal audiological expectations are realized.

It may be that the present data merely indicate that the current generation of multichannel implants can have salutary audiological and psychological influences. Although the present study does not clearly establish the link between audiological benefit and psychological benefit, the demonstration of psychological improvement following 18 months of implant use is encouraging and strongly suggests that implants can have positive influences on the behavioral and emotional status of persons with acquired profound hearing loss.

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EFFECT OF AGE AT ONSET OF DEAFNESS ON CHILDREN'S SPEECH PERCEPTION ABILITIES WITH A COCHLEAR IMPLANT

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The speech perception abilities of 37 children with cochlear implants (single-channel or multichannel) were examined as a function of age at onset of deafness. There was no significant difference in the speech perception abilities of implanted children who were born deaf and those of implanted children who lost their hearing during the first 3 years of life. In contrast, the performance of children whose age at onset of deafness was 5 years or later was significantly better than that of the children with congenital or early-acquired deafness on tests of stress pattern categorization, closed-set word identification, open-set identification of common phrases, and lipreading enhancement.

KEY WORDS — cochlear implant, onset of deafness, speech perception.

It is evident from research findings that profoundly hearing-impaired children, who receive negligible benefit from hearing aids, can derive benefit from a cochlear implant.[1-3] There are, however, large individual differences in the speech perception abilities among children who use a single-channel or multichannel cochlear implant.[1-3] As more data become available on the performance of implanted children, it is possible to identify factors that account for the variance among subjects. This information has important implications for counseling parents and helping them develop realistic expectations about potential benefits that their child might receive from an implant.

One obvious factor to consider is age at onset of deafness. A common notion among clinicians and researchers is that children who experience some period of normal auditory development before the onset of deafness will demonstrate higher levels of performance with an implant than will children who are born deaf. This notion is based on research findings that show that normal-hearing infants discriminate speech sounds according to phonetic categories,[4-6] and that during the first year of life, they begin to perceive only those sound differences that are used phonemically in their native language in a manner similar to that demonstrated by adults.[7,8] It has been hypothesized that with linguistic experience, infants learn to attend selectively to those contrasts that are phonologically relevant in their language and to disregard those that are not.[7,8] Given the evidence that infants process speech in a linguistically meaningful way during the first year of life, it might be hypothesized that they also develop and store auditory images of the relevant acoustic contrasts of their language. If this is so, then children with acquired deafness might achieve higher performance levels with an implant than children with congenital deafness because they might retain these auditory images to later help them decode the information perceived via an implant.

It is unclear, however, how much prior experience with language an individual needs to demonstrate a perceptual advantage with an implant over an individual who has had little or no linguistic experience prior to the onset of deafness. Available data suggest that children with onset of deafness around 5 years of age show higher performance levels with the Nucleus 22-channel implant than do children with congenital or early-acquired losses.[2,9-11] Few data have been reported on performance differences between children who are born deaf and those who lose their hearing during the first 3 years of life. The purpose of the present investigation was to examine the effect of age at onset of deafness on the speech perception abilities of children who use a single-channel or multichannel cochlear implant.

METHODS

Subjects. Thirty-seven profoundly hearing-impaired children who used either a single-channel (3M/House) or multichannel (Nucleus 22-channel) cochlear implant were grouped according to four categories of age at onset of deafness. Group 1 (n = 12) consisted of children who were born deaf or whose onset of deafness occurred during the first year of life. Group 2 (n = 13) consisted of children whose onset of deafness occurred between 1.1 and 2.0 years of age. Group 3 (n = 6) consisted of children whose deafness occurred between 2.1 and 3.5 years of age, and group 4 (n = 6) consisted of children whose onset of deafness occurred between 3.6

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TABLE 1. SUBJECT CHARACTERISTICS

Subject	*Age at Onset (y)*	*Age at Implant Fitting (y)*	*Years of Deafness*	*Years of Implant Use*	*Type of Implant*
Group 1 (n = 12)					
SDL	0.5	5.4	4.9	1.0	Nucleus
SAF	0.0	4.5	4.5	5.5	3M/House
SBE	0.5	3.3	2.8	2.5	3M/House
SAD	0.0	4.9	4.9	1.5	Nucleus
SAK	0.3	7.2	6.9	2.0	Nucleus
SDI	0.0	6.7	6.7	1.0	Nucleus
SAE	0.0	2.5	2.5	3.0	3M/House
SAG	0.0	3.0	3.0	3.5	3M/House
SBU	0.0	11.1	11.1	2.0	Nucleus
SDF	0.0	12.0	12.0	0.5	Nucleus
SAI	0.8	11.5	10.7	4.0	3M/House
SCH	0.0	12.5	12.5	3.0	3M/House
Mean	0.2	7.0	6.9	2.5	
SD	0.3	3.8	3.8	1.4	
Group 2 (n = 13)					
SDM	1.9	5.3	3.4	0.5	Nucleus
SBJ	1.3	3.9	2.6	2.5	3M/House
SBR	1.3	4.5	3.2	2.5	3M/House
SDB	2.0	6.0	4.0	2.5	3M/House
SCU	2.0	10.1	8.1	1.0	3M/House
SCA	1.8	4.3	2.5	2.0	Nucleus
SDC	1.7	6.6	4.8	1.0	Nucleus
SAJ	1.3	10.1	8.8	1.0	3M/House
SBO	1.6	8.7	7.1	4.5	3M/House
SAL	1.5	4.1	2.6	4.5	Nucleus
SBN	1.3	7.1	5.8	1.5	Nucleus
SDP	1.5	13.0	11.5	1.5	Nucleus
SBL	1.5	10.8	9.3	4.0	3M/House
Mean	1.6	7.3	5.7	2.2	
SD	0.3	3.0	3.0	1.4	
Group 3 (n = 6)					
SDA	2.8	5.3	2.5	2.5	3M/House
SCQ	2.8	6.3	3.5	1.5	Nucleus
SCD	2.3	12.9	10.6	3.5	3M/House
SDN	2.5	5.8	3.4	1.5	Nucleus
SDS	3.5	9.2	5.7	0.5	Nucleus
SCO	2.8	13.2	10.4	1.0	Nucleus
Mean	2.8	8.8	6.0	1.8	
SD	0.4	3.6	3.6	1.1	
Group 4 (n = 6)					
SBC	5.7	7.3	1.6	5.5	3M/House
SBZ	5.3	6.3	1.0	3.0	3M/House
SCI	6.7	8.0	1.3	1.5	3M/House
SDO	6.3	7.3	1.0	1.0	Nucleus
SDR	4.2	5.2	1.0	1.0	Nucleus
SBY	6.0	11.5	5.5	5.5	3M/House
Mean	5.7	7.6	1.9	2.9	
SD	0.9	2.1	1.8	2.1	

and 6.0 years of age.

Relevant background information on the subjects appears in Table 1. Eight of the subjects in group 1 had congenital hearing losses and another three subjects became deaf before 6 months of age. Only one subject in group 1 became deaf after 6 months of age. The onset of hearing loss in most of the subjects in group 4 occurred at 5 years of age or later, and there was only one subject in the sample whose onset of deafness occurred between 3.6 and 5.0 years of age. The cause of deafness in children with acquired losses was meningitis, with the exception of two children in group 4, who had progressive hearing losses (subjects SDO and SDR). The size of groups 3 and 4 is smaller than that of the other two because of the lower incidence of meningitis after 2 years of age.

Separate one-way analyses of variance (ANOVAs) revealed no significant group difference in length of implant use ($F_{3,36} = 0.66$, $p = .583$) or the age at which the subjects received an implant ($F_{3,36} = 0.41$; $p = .743$). There was, however, a significant group difference in the duration of deafness before implantation ($F_{3,36} = 3.27$, $p = .03$) due to the shorter period of deafness of the subjects in group 4 than in the other three groups. This difference is explained by the trend to implant children with a later onset of deafness much sooner than children with early-acquired or congenital deafness.

Performance was not analyzed in terms of type of implant because of an insufficient number of subjects in each group with each type of device. Because pediatric users of both types of devices demonstrate a wide range of speech perception abilities, it was appropriate to combine users of the two devices within a group.[1] The number of subjects with each type of device was nearly equal in each group (Table 1). All subjects received an implant because they derived little or no benefit from conventional hearing aids.

Speech Perception Measures. The children's performance was assessed on a range of speech perception measures with auditory cues only or with combined auditory plus visual cues. Data are reported for each child's most recent postimplant evaluation session, which corresponds to number of years of implant use in Table 1.

The Change/No Change test[12] is designed to assess detection of a change in a segmental or suprasegmental feature of speech. The paradigm is based on that developed to assess infant speech perception abilities. There are nine subtests, each with one feature contrast: syllable length (long versus short), intonation (statement versus question), fundamental frequency (steady versus changing), talker gender (male versus female), vowel height (high vowel versus low vowel), vowel place (front vowel versus back vowel), consonant manner (stop versus fricative), consonant voicing (voiced versus voiceless), and consonant place (front versus back). A subtest consists of 15 trials, with each trial consisting of a string of 10 nonsense syllables spoken by a male talker. For a no-change trial, the 10 stimuli are the same. For a change trial, the last 5 stimuli differ from the first 5. The stimuli were digitized and manipulated to equate the overall duration and intensity of the syllables within a trial. The stimuli were audiotaped and presented in the sound field at a

TABLE 2. SCORES ON CHANGE/NO CHANGE SUBTESTS

Subtest	*Group 1*	*Group 2*	*Group 3*	*Group 4*
Change/No Change[12] composite				
Mean (%)	71	71	74	81
SD	19.8	9.9	12.7	13.7
Syllable length				
Mean (%)	82	88	91	97
SD	22.2	16.0	9.1	5.5
Intonation				
Mean (%)	59	60	69	69
SD	15.7	18.7	19.6	26.6
Fundamental frequency				
Mean (%)	80	75	73	89
SD	21.7	16.8	22.7	15.0
Gender				
Mean (%)	72	65	67	89
SD	26.2	23.5	24.2	13.3
Vowel height				
Mean (%)	71	82	87	89
SD	31.3	15.7	19.5	19.6
Vowel place				
Mean (%)	69	71	60	84
SD	28.5	17.8	30.6	18.9
Consonant voicing				
Mean (%)	57	50	63	63
SD	18.3	10.1	11.9	14.2
Consonant manner				
Mean (%)	78	76	80	83
SD	20.4	12.9	15.0	22.4
Consonant place				
Mean (%)	70	68	79	71
SD	23.6	18.3	14.4	19.9

level of 70 dB sound pressure level. The child was trained to respond when a change trial occurred. Chance level of performance is 50%.

The Monosyllable-Trochee-Spondee (MTS) test[13] is administered live-voice. It consists of 12 pictured nouns with three different stress patterns. Each item is presented two times in random order, and the subject's score is averaged across the two trials. Each response is scored for 1) stress-pattern categorization and 2) word identification. Chance level of performance is 33% on the stress-pattern categorization task and 8% on the word identification task.

The Minimal Pairs test[1] consists of pairs of pictured words, with members of a pair differing in terms of vowel height, vowel place, consonant manner, consonant voice, or consonant place. There are 80 items, and performance is expressed in terms of the percentage of total number of items correctly identified. The child points to the picture corresponding to the item presented live-voice by the examiner. Chance level of performance is 50%.

The Hoosier Auditory Visual Enhancement (HAVE) test[1] is used to evaluate the integration of auditory and visual information. The test consists of three pictured items. Two items are visually similar (eg, *man* and *pan*) on the lips whereas the remaining one is visually distinct (eg, *fan*). On a given trial, only one of the visually similar words is presented but always with combined auditory and visual cues. Each response is scored on the basis of visual correctness (HAVE:Visual; ie, the child selects either one of the visually similar words) and word correctness (HAVE:Word; ie, the child correctly selects the target because both auditory and visual cues are perceived accurately). Chance level of performance is 33%.

The Common Phrases test[1] assesses understanding of familiar phrases used in everyday situations. A modified open-set format is used with pretest familiarization of item topics. There are six lists with 10 phrases each that are either simple commands or questions. The phrases are presented in two conditions: auditory-only and auditory-plus-visual cues. The test is always administered in the auditory-plus-lipreading condition first. If the child does not correctly repeat at least 2 of the phrases when visual cues are present, testing is not performed in the auditory-only condition, and the child is assigned a score of zero for that condition. Performance is scored in terms of the percentage of phrases correctly repeated by the child.

The Connected Discourse Tracking Procedure[14] has been modified for use with our subjects who have severely delayed language abilities. One of three levels of material is used: three-sentence stories with accompanying pictures, multiple-paragraph stories with accompanying pictures, and story-length text without pictures. The subject is required to repeat (spoken or signed) verbatim what is said by the clinician. When breakdowns occur, the clinician repeats the information once verbally, then adds prompts (ie, a sign, finger-spelling, cueing, or written word, depending on the child's mode of communication) one word at a time. Each subject tracked in 5-minute blocks under two conditions: lipreading alone (with the implant off), and lipreading with the implant on, with the order of conditions alternated across subjects.

Tracking data were analyzed in terms of the number of words tracked per minute under each condition, and the difference in tracking rate between the two conditions. The latter measure reflects the amount of lipreading enhancement provided by the implant. Some children could not perform even the simplest tracking task because of their limited language and/or perception skills. For some, the tracking procedure had not been developed at the time that they were tested. Therefore, data are not available for all subjects on this measure.

RESULTS

The scores for the subjects in the four groups on each test were subjected to a one-way ANOVA with group as the main factor. For the Change/No

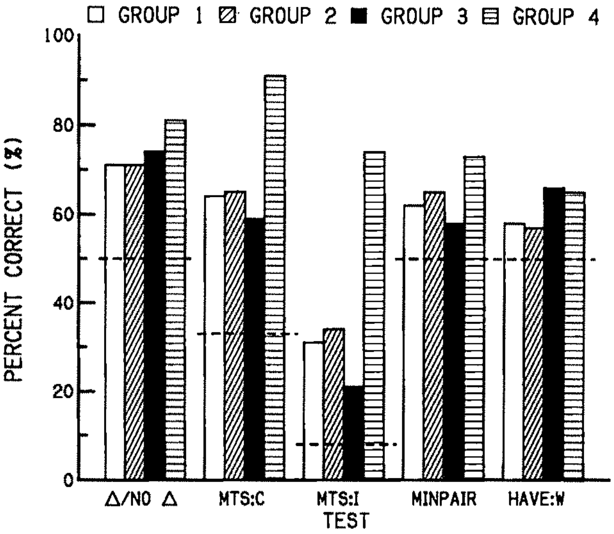

Fig 1. Mean scores for subjects with cochlear implants with age at onset of deafness between birth and 1.0 year of age (group 1; n = 12), at 1.1 to 2.0 years of age (group 2; n = 13), at 2.1 to 3.5 years of age (group 3; n = 6), or after 3.6 years of age (group 4; n = 6) on closed-set test of auditory discrimination (Change/No Change[12]; Δ/NO Δ), stress-pattern categorization (MTS:C),[13] word identification (MTS:I),[13] feature identification (MINPAIR),[1] and auditory-visual feature integration (HAVE:W).[1] Dashed lines — chance levels of performance.

Change test data, a separate ANOVA was performed on the composite score (average of the nine subtests) and on the score of each of the nine subtests. The results of the ANOVAs revealed no significant difference among the groups on the composite score of the Change/No Change test, or on any of the nine subtests. The mean scores for each group of subjects on the Change/No Change test appear in Table 2.[12] The composite score for the four groups of subjects on the Change/No Change test also is illustrated in Fig 1.[1,12,13]

Mean scores on the MTS, Minimal Pairs, and HAVE also appear in Fig 1. The results of the one-way ANOVA on the MTS data revealed a significant group effect on the stress-pattern categorization task of the MTS ($F_{3,33} = 3.42$, $p < .05$). Post hoc pairwise comparisons with the Scheffe test, however, revealed no significant differences between groups.

There was a significant group difference on the MTS word identification ($F_{3,33} = 7.92$, $p < .01$). Post hoc analysis with the Scheffe test revealed that the performance of the subjects in group 4 differed significantly ($p < .01$) from that of the other three groups of subjects. All other group comparisons were nonsignificant.

The performance of the four groups was similar on the Minimal Pairs test, as shown in Fig 1. The results of the ANOVA revealed a nonsignificant group effect ($F_{3,33} = 2.05$, $p = .669$). A similar pattern of performance was observed for the HAVE:Word score, which requires integration of auditory and visual features ($F_{3,33} = .52$, $p = .669$). The mean scores for the HAVE:Visual are not shown because all groups obtained high scores on this measure (mean for group 1, 98%; group 2, 97%; group 3, 100%; and group 4, 93%).

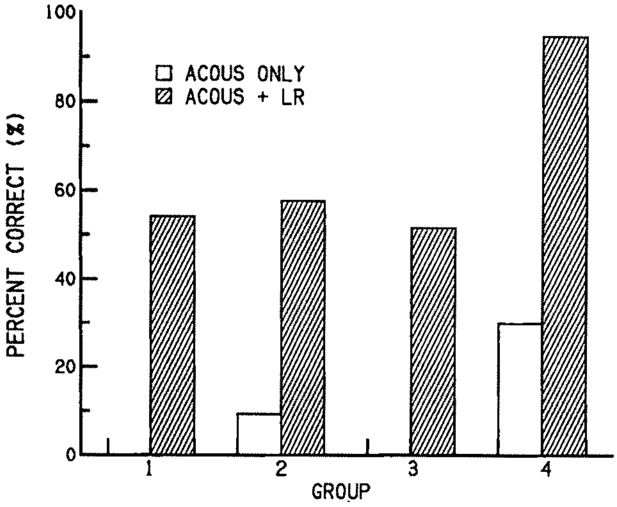

Fig 2. Mean scores for subjects with cochlear implants with age at onset of deafness between birth and 1.0 year of age (group 1; n = 12), at 1.1 to 2.0 years of age (group 2; n = 13), at 2.1 to 3.5 years of age (group 3; n = 6), or after 3.6 years of age (group 4; n = 6) on test assessing recognition of common phrases using modified open-set format.[1] ACOUS — acoustic, LR — lipreading.

The mean scores of the four groups on the Common Phrases test is illustrated in Fig 2.[1] The results of the one-way ANOVA on the data collected in the auditory-only condition revealed the group effect to be statistically significant ($F_{3,33} = 3.83$, $p < .05$). Post hoc group comparisons with the Scheffe test revealed that the performance of the subjects in group 4 differed significantly ($p < .05$) from that of the subjects in group 1. All other group comparisons were nonsignificant. The ANOVA results performed on the data collected in the auditory-plus-lipreading condition revealed no significant group differences ($F_{3,33} = 1.84$, $p = .162$).

The tracking data are shown in Fig 3.[14] The main effect of group was nonsignificant in the lipreading-alone condition ($F_{3,25} = 2.12$, $p = .123$). In contrast, there was a significant group effect in the lipreading-plus-device condition ($F_{3,25} = 5.32$, $p < .01$). The results of the Scheffe test revealed that the performance of group 4 differed significantly from that of group 1 ($p < .01$) and group 2 ($p < .05$).

To obtain a measure of degree of lipreading enhancement with the implant, the difference between the tracking rates with and without the device was examined. A difference score calculated by subtracting the tracking rate in the lipreading-plus-device condition from that obtained in the lipreading-only condition was obtained for each subject. This difference score was then subjected to a one-way ANOVA with group as the main factor. The results

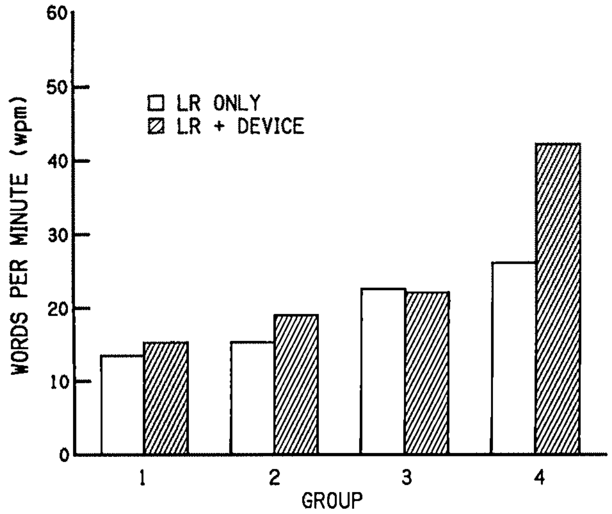

Fig 3. Mean tracking[14] rate, expressed in words per minute, for subjects with cochlear implants with age at onset of deafness between birth and 1.0 year of age (group 1; n = 8), at 1.1 to 2.0 years of age (group 2; n = 11), at 2.1 to 3.5 years of age (group 3; n = 5), or after 3.6 years of age (group 4; n = 5). Tracking was performed by using lipreading (LR) only and lipreading plus implant for period of 5 minutes in each condition.

revealed a significant group effect ($F_{3,25} = 6.34$, $p < .01$). Post hoc analysis with the Scheffe test revealed a significant difference between the performance of the subjects in group 4 and that of the subjects in group 3 ($p < .01$), and between the performance of the subjects in group 4 and that of the subjects in groups 1 and 3 ($p < .05$).

DISCUSSION

The results of this study showed no significant difference in the speech perception abilities of children with cochlear implants who were born deaf or who acquired their deafness during the first 3 years of life. A finding of significance is the performance similarity of these children on all measures that were designed to assess a wide range of speech perception skills. Thus, it appears that early auditory experience (ie, 1 to 3 years) provides no obvious advantage in learning to perceive and interpret information transmitted by a cochlear implant. On the other hand, these data suggest that children who are born deaf have the potential to derive as much benefit from a cochlear implant as do children who acquire their deafness during the first 3 years of life.

The only group that obtained significantly higher scores than the other groups was group 4, which comprised mainly children with onset of deafness at or after 5 years of age. This group of subjects achieved significantly higher scores than did the other groups on MTS stress-pattern categorization, MTS closed-set word identification, open-set identification of phrases (Common Phrases), and enhancement of lipreading skills (connected discourse tracking). Their performance was similar to that of the other subjects on auditory discrimination of speech features (Change/No Change test) and on tests involving identification of words differing in only one phonetic feature on the basis of only auditory cues (Minimal Pairs test) or combined auditory and visual cues. The lack of significant difference between groups on the feature discrimination task (Change/No Change test) is due to the relatively high scores obtained by all groups on this task. In contrast, all groups obtained relatively low scores on the feature identification tasks.

It is important to note that performance as a function of age at onset of deafness might be confounded by cause of deafness in this study. The hearing losses of all subjects in the three younger groups with acquired losses were due to meningitis. It is well documented that meningitis can result in other cognitive and neurologic deficits that might affect performance on speech perception tests.[15] Also, cochlear ossification often is a consequence of meningitis that can adversely affect performance with an implant.[16] Thus, children whose deafness is due to meningitis lose access to auditory input, and in addition, they are at risk for the other consequences of this disease that might affect their performance with an implant. In contrast, children with progressive hearing losses probably demonstrate only the consequences due to loss of auditory information. Moreover, most of these children do not become implant candidates until they are at least 5 years of age, when their hearing loss has progressed to the profound range of impairment. By the time that these children lose access to acoustic speech information, they may have had sufficient time to establish and maintain critical auditory and speech skills.

The results of this study are in general agreement with those of other investigations with respect to the superior performance of children with a late onset of deafness (ie, 5 years of age or later).[2,9-11] The findings of Staller et al[2] indicate some difference in the perception abilities of children with congenital deafness and those of children with early-acquired deafness. However, Staller et al classified children in terms of length of deafness as well as age at onset of deafness, so direct comparison of their findings to those of the present study is not possible. The data of Staller et al did show that the performance differences between the children with congenital deafness and those with early-acquired deafness were much smaller than the differences between the children with late onset of deafness and those with congenital or early-acquired deafness.

In summary, the results of the present investigation reveal that children who heard normally for 5 years or more before becoming deaf achieved significantly higher speech perception scores with an implant than did children with an earlier onset of deafness. In contrast, there appears to be no obvious difference in the speech perception abilities of children with implants who were born deaf or who experienced a short period of normal auditory development (ie, 1 to 3 years) before the onset of their deafness. Future research is needed to determine if this trend continues when the performance of only multichannel implant users is examined.

ACKNOWLEDGMENTS — The assistance of Wendy Myres, Kathy Kessler, and Molly Pope in the management of the children and Leslie K. Sam in the data analyses is gratefully acknowledged. Michael A. Novak, MD, and Jill Firszt, MA, of the Carle Clinic, Champaign, Illinois, and John L. Kemink, MD, and Susan Zimmerman-Phillips, MS, are gratefully acknowledged for allowing us to evaluate several of the children implanted at their centers.

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ROUND WINDOW VERSUS PROMONTORY STIMULATION: ASSESSMENT FOR COCHLEAR IMPLANT CANDIDACY

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Electrical stimulation of the cochlea is a part of the preliminary evaluation protocol for cochlear implant candidacy at many centers. Typically, a transtympanic needle electrode is placed on the promontory of the ear under consideration and electrical current is presented to measure auditory percepts and psychophysical-electrophysiologic measures. Since 1984, we have used a ball electrode placed directly on the round window (via a tympanotomy) for stimulation, with 27 subjects being so tested to date. Nine additional subjects have had stimulating electrodes placed on both the round window and the promontory. Seven of these subjects yielded complete data. For measurements of threshold, maximum acceptable loudness level, and dynamic range, promontory testing mean values were higher than round window measurements. However, the differences were not significant.

KEY WORDS — cochlear implant, promontory, round window.

INTRODUCTION

Electrical stimulation of the cochlea prior to cochlear implantation has become a routine part of the candidate selection protocol at many centers. The procedure is performed in the belief that it assesses the degree of surviving neural components and hence the stimulability of the cochlear nerve fibers in profoundly deafened individuals. Positive results are felt to be predictive of auditory perception with a cochlear implant. Both subjective and objective tests have been used. These include simple psychophysical procedures such as threshold of audibility and dynamic range[1] and gap detection thresholds,[2] as well as electrophysiologic procedures such as electrically evoked auditory potentials, in-

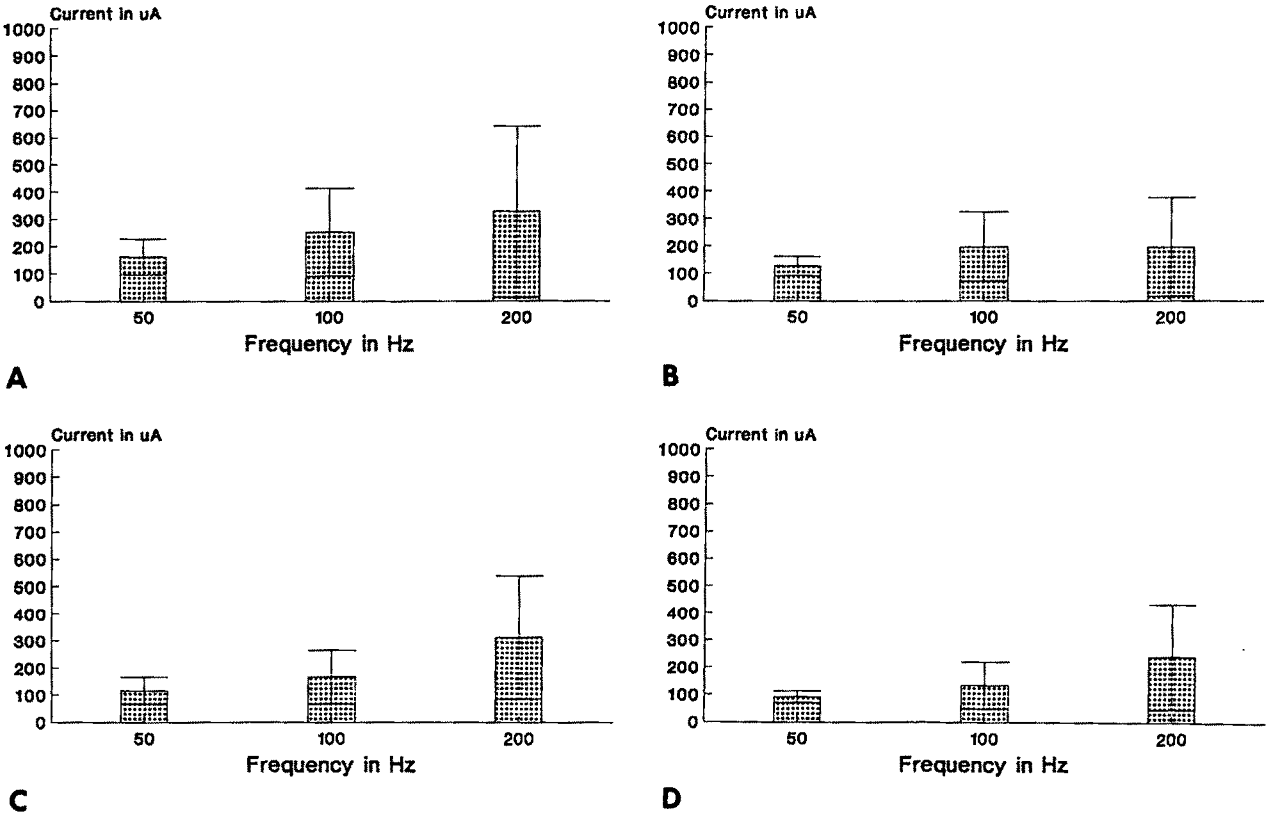

Fig 1. Means ± 1 SD for thresholds (n = 7 in each part, A-D). A) Promontory, sine wave. B) Round window, sine wave. C) Promontory, square wave. D) Round window, square wave.

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RESULTS OF *T* TESTS COMPARING PROMONTORY AND ROUND WINDOW

	t	df	p^*
Thresholds			
Square waves			
50 Hz	1.05	6	NS
100 Hz	1.15	6	NS
200 Hz	1.24	6	NS
Sine waves			
50 Hz	1.21	6	NS
100 Hz	1.19	6	NS
200 Hz	1.13	6	NS
Maximum acceptable loudness levels			
Square waves			
50 Hz	1.32	6	NS
100 Hz	1.11	6	NS
200 Hz	1.30	6	NS
Sine waves			
50 Hz	1.06	6	NS
100 Hz	1.37	6	NS
200 Hz	1.38	6	NS
Dynamic range			
Square waves			
50 Hz	1.58	6	NS
100 Hz	1.02	6	NS
200 Hz	0.49	6	NS
Sine waves			
50 Hz	1.43	6	NS
100 Hz	1.34	6	NS
200 Hz	1.48	6	NS

*p level of .01 was considered significant.

cluding auditory brain stem responses (ABRs)[3,4] and middle latency responses.[5] Although it is hoped that one or more of these procedures will yield prognostic indicators, none have correlated well with postimplant speech perception skills.[1]

House and Brackmann[6] reported on the results of promontory stimulation with a transtympanic needle electrode such as that used in electrocochleography with a large group of profoundly deaf implant candidates. They suggested that the procedure could be used as a means of distinguishing between sensory and neural deafness and confirming a subject's suitability for implantation before operation. Since that time, a transtympanic needle electrode placed on the promontory has become the standard practice at many centers. The rationale for this method has been that electrode placement is easy and the procedure is only mildly invasive.

The round window has also been proposed as a site for electrical stimulation; however, the placement of the electrode is a more invasive procedure. Very little research has been performed to compare results between the promontory and round window stimulation sites. Lusted et al[7] reported on the results of promontory, round window, and scala tympani stimulation for the recording of electrical ABRs with a group of deafened cats. Their results revealed that in the case of light to medium ganglion cell loss, the scala tympani site provided the steepest input-output function for ABR amplitude. Round window results were present at higher thresholds, and smaller peak amplitudes with a more gradual input-output function and promontory results were worse or even absent. For ears with less than 25% ganglion cell survival, scala tympani and round window results were markedly reduced but comparable, while promontory results were very poor or absent.

Since 1984, we have placed a 2-mm ball electrode directly on the round window via a tympanotomy.[8] Our first 27 implant candidates were assessed with only the round window–stimulating electrode. To determine if differences existed between round window and promontory stimulation, a study was proposed whereby candidates at our center would have electrodes placed on both of these sites. This paper presents data for threshold (T), maximum acceptable loudness (MAL) levels, and resulting dynamic range (DR) for each electrode site for constant current sine and square waves at 50, 100, and 200 Hz.

METHOD

Subjects. Nine adult cochlear implant candidates served as subjects in this study. All had severe to profound sensorineural hearing losses of postlingual onset. There were four men and five women with an average age of 44.0 years (SD = 15.41 years). The average duration of profound deafness was 16.0 years (SD = 19.45 years). The onset of deafness was sudden for four subjects and progressive for five subjects. Five subjects (56%) were deaf from unknown causes, three (33%) from meningitis, and one (11%) from Meniere's disease.

Procedure. The middle ear is accessed through a transcanal tympanomeatal incision. The bony overhang overlying the round window membrane is removed and a 2-mm ball electrode is placed directly on the round window. The tympanomeatal flap is replaced after the electrode is sutured along the posterior canal wall. A myringotomy incision is then made and another electrode is seated into a small niche drilled into the middle portion of the cochlear promontory. This electrode is secured by a suture to the anterior canal wall. Both electrodes are further secured by sutures to the pinna. The ear is dressed and the electrode leads are positioned behind the pinna to allow access for connection to the stimulating equipment.

For 3 days following the operation, subjects underwent a variety of psychophysical and electrophysiologic tests. The T, MAL, and DR data presented in this paper were obtained for each electrode site over four sessions. The averaged values for each site, stimulus type, and frequency for each subject were entered into the data base. Electrode leads were color-coded and assigned by the surgeon to either the promontory or the round window

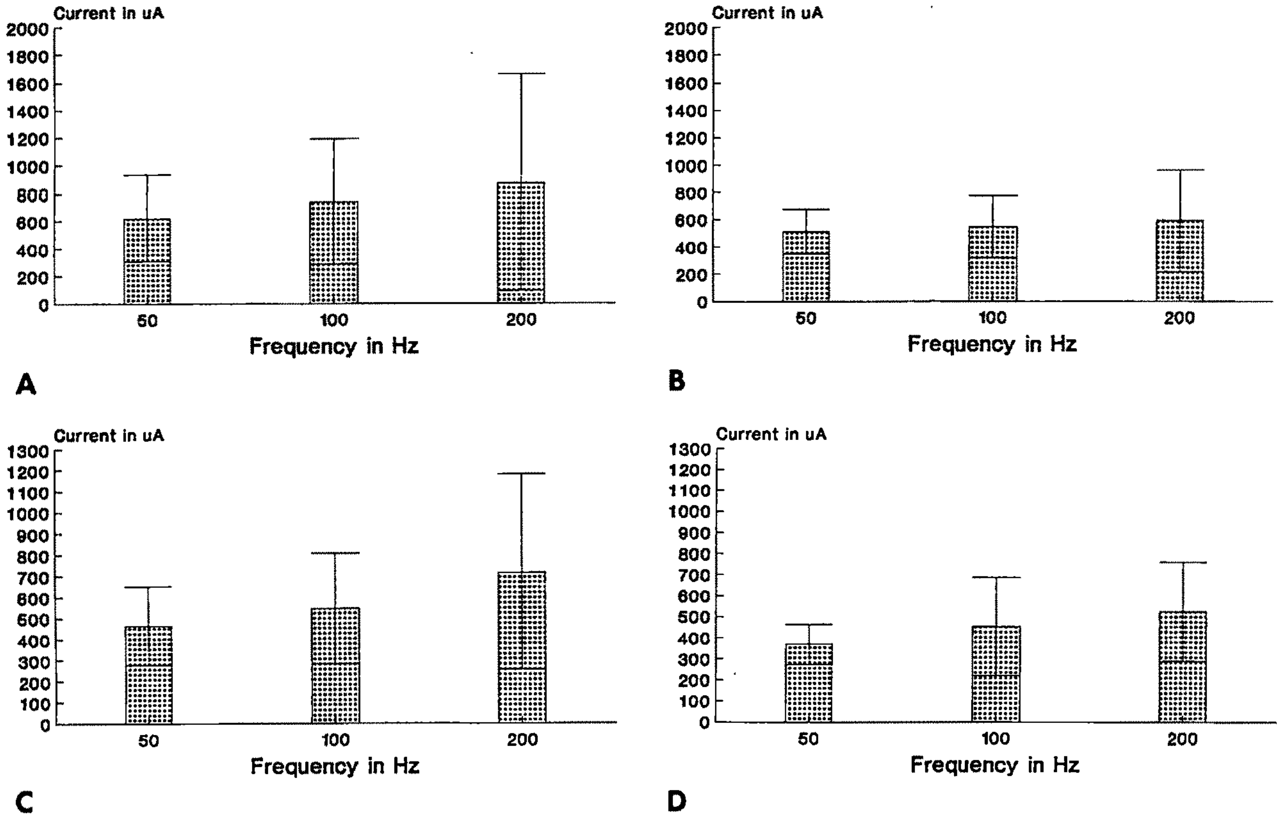

Fig 2. Means ±1 SD for maximum acceptable loudness levels (n = 7 in each part, A-D). A) Promontory, sine wave. B) Round window, sine wave. C) Promontory, square wave. D) Round window, square wave.

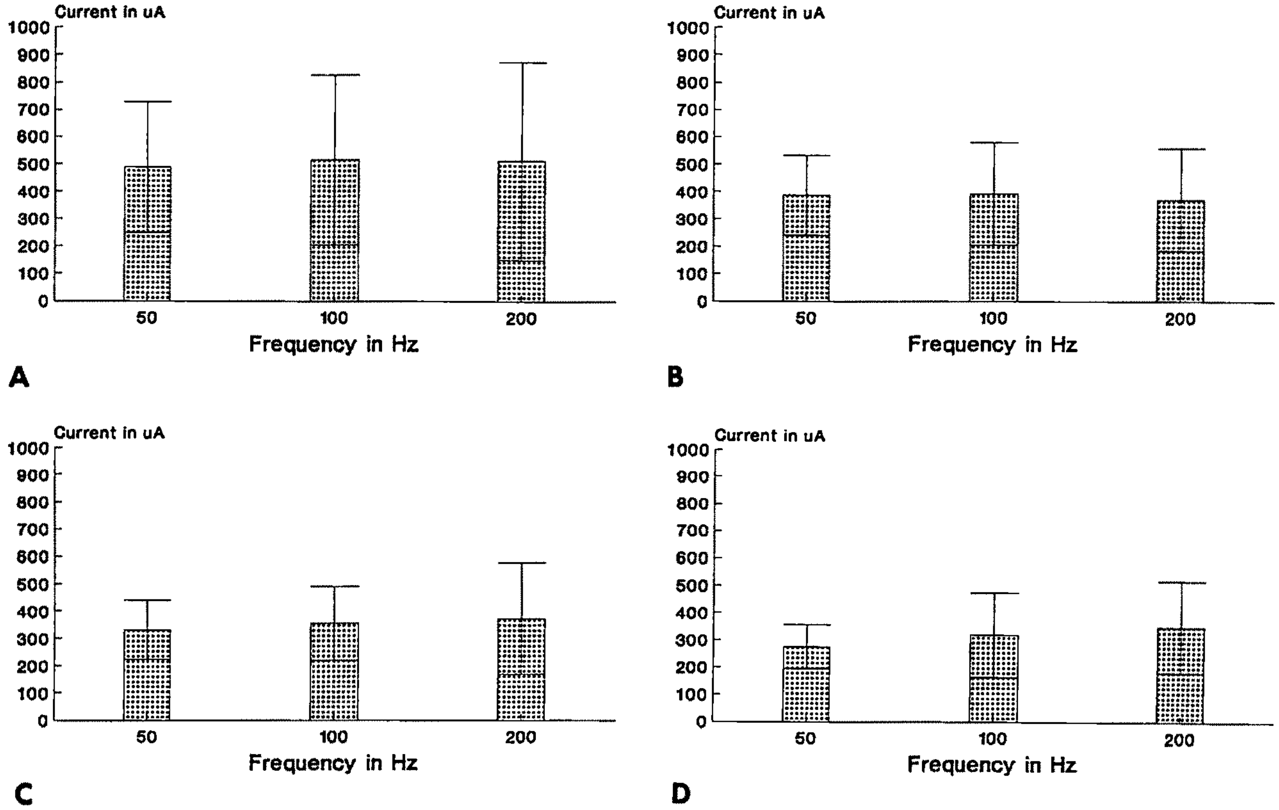

Fig 3. Means ±1 SD for dynamic range (n = 7 in each part, A-D). A) Promontory, sine wave. B) Round window, sine wave. C) Promontory, square wave. D) Round window, square wave.

through a randomization table. The order of electrode testing for each session was determined by a randomization table, as well. The tester was blinded as to the origin of each electrode lead until the study was completed. Stimulating equipment consisted of a stimulus generator (Wavetek model 105 5-MHz lin-log sweep generator), a control unit (controls stimulus amplitude, duration, rise-fall time, etc), and a bioelectric stimulus isolator (Coulbourn E13-72).

RESULTS

Figure 1A,B shows the results for average T levels and standard deviations for sine waves for promontory and round window testing. Figure 1C,D shows average T levels and standard deviations for square waves for promontory and round window data. The results show the mean values for promontory data to be higher for both stimuli at all frequencies; however, the differences between stimulating sites were not significant (see Table).

Figure 2A,B shows the results for sine waves and Fig 2C,D shows the results for square waves for promontory and round window MAL measurements. The results again show higher mean values for promontory data, but the differences were not significant (see Table).

Figure 3A,B shows the results for sine waves and Fig 3C,D shows the results for square waves for promontory and round window DR measurements. Results for promontory testing were again higher than for round window results; however, the differences were not significant (see Table).

It should be noted that the data presented here are for 7 of the 9 subjects. One subject experienced only an "electrotactile" sensation from both electrodes. Another was stimulated well with the round window electrode but was not stimulated with the promontory electrode. This occurred despite no significant difference in electrode impedances. Of the 36 subjects seen to date in our program, only these 2 and 1 other have failed to experience an auditory sensation with the stimulation.

Despite the seemingly large differences in mean values between promontory and round window data for some measurements, there were no statistically significant differences between the values. The standard deviations, as observed in the Figures, were large, especially for the promontory data. This, along with the small number of subjects to date in this study, likely accounts for the lack of significance.

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INDICATION OF A RELATION BETWEEN SPEECH PERCEPTION AND TEMPORAL RESOLUTION FOR COCHLEAR IMPLANTEES

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In order to better understand the reasons for success or failure of a cochlear implant system for various patients, it appears necessary to analyze the patients' basic psychophysical capacities in relation to speech perception. Five patients with intracochlear multichannel Ineraid implants were studied in terms of their performance on temporal analysis in relation to their perception of consonants. For temporal analysis we measured the detection of a silent gap in noise and of an interval between two clicks. For consonant perception we established a confusion matrix based on 12 consonants presented in a vowel-consonant-vowel context using the vowel /a/. The results showed a correlation between temporal resolution for two successive clicks at the most basal cochlear electrode used, and the perception of place of articulation of consonants. This finding indicates that delivering fine temporal coding can be crucial for the success of an implant and that for a given subject, optimal capacity for temporal resolution may serve as a criterion for choosing a basal electrode.

KEY WORDS — cochlear implant, speech perception, temporal resolution.

INTRODUCTION

It has been clearly established for several years that some multichannel cochlear implant systems give overall much better results than single-channel systems.[1-4] For a given multichannel system, however, performances vary considerably among patients. Trials designed to discover the reasons underlying such a variability have given only moderate clues, and the prognosis of success remains very poor. For instance, in a previous series of investigations we did not find any relation between speech performance and some basic psychophysical abilities for patients implanted with an Ineraid cochlear implant.[5] The principal objective of cochlear implants is to restore speech understanding, and it is well known that the acoustic features of speech sounds are specific spectral configurations changing rapidly over time.[6] The consonants in particular differ greatly in their temporal structure.[7,8] It was the objective of this study to investigate whether subjects presenting various degrees of consonant identification would show related variations in performance on temporal resolution. Several previous studies examined the capacities of temporal resolution with electrical stimulation of the cochlea and found them equivalent to those of normally hearing individuals for acoustic stimuli,[9-13] but they did not examine their relation to speech perception.

MATERIALS AND METHODS

Five postlingually deafened patients were the subjects in these experiments. They were all implanted with an Ineraid multichannel system. The existence of a percutaneous pedestal allowed direct access to and stimulation of each electrode. Of the six available electrodes, only two were used for the tests: the most apical and the most basal of those normally used by the subjects with their processors. Direct stimulation was performed with an optically isolated current generator. Similar to the normal functioning mode of the Ineraid, stimuli were delivered between one intracochlear electrode and a common ground electrode in the temporal muscle. On the two intracochlear electrodes tested we measured absolute perception thresholds and temporal resolution. For absolute threshold determination we used frequencies at octave intervals between 50 and 3,200 Hz. Two tests of temporal discrimination were employed. In the first a silent gap in the middle of 500 milliseconds of white noise was used; in the second two successive negative-going impulses of 100 microseconds were used, separated by an adjustable interval. All measures of temporal resolution were performed at a comfortable level. For the first test the subjects were asked to detect the presence of an interruption in the noise. For the second test they were presented alternately with one click or two clicks and they were asked to detect, not a clear silent separation between the two clicks, but the earliest change in sensation that they described as going from "toc" to "troc." The consonant recognition test was based on videotape recordings from University College London. It consisted of a series of 12 consonants (b, p, d, t, k, g, f, v, s, z, m, and n) uttered in a vowel-consonant-vowel context using the vowel /a/ and presented in random order. Series of recordings were available from different male

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From the Laboratory of Experimental Audiology, Pellegrin Hospital, Bordeaux, France (Cazals), and the Otorhinolaryngology and Head and Neck Surgery Clinic, Cantonal University Hospital, Geneva, Switzerland (Pellizone, Kasper, Montandon). Supported by a grant from Region Aquitaine (8903049).

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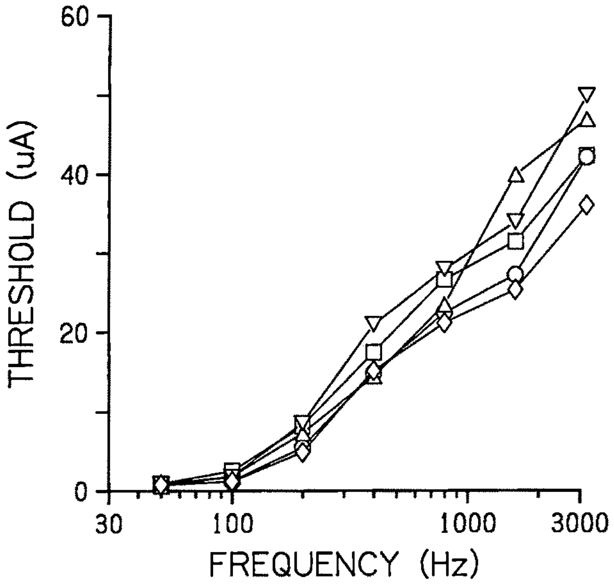

Fig 1. Threshold values in microamperes at different octave frequencies for most basal electrode normally used by five subjects of study.

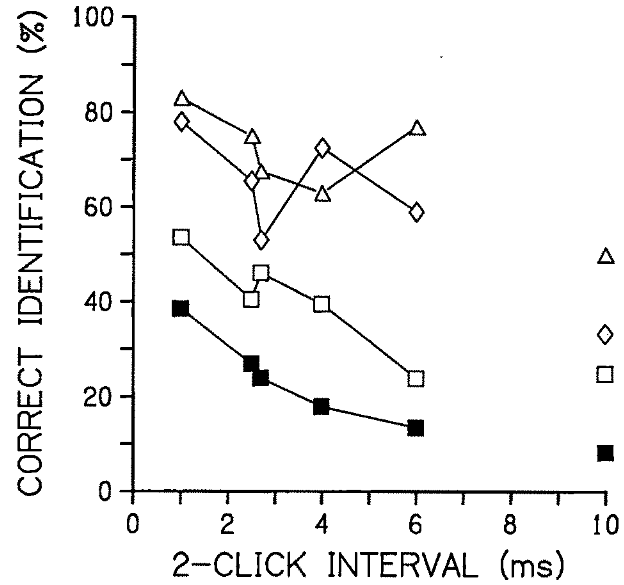

Fig 2. Identification scores, averaged for male and female voices, for each patient as function of temporal resolution at basal electrode for two-click test. Each group of symbols at same place on abscissa corresponds to results of one subject. At 10 milliseconds on abscissa each symbol shows level of chance score. Black squares — global, open squares — place of articulation, diamonds — mode of articulation, triangles — voicing.

and female speakers, and stimuli were presented in free field at a level of about 80 dBA at the patient's ear. All tests of consonant recognition were performed with the subjects wearing their Ineraid system in its usual setup. Each subject was tested with a male and a female voice, and the results were analyzed as scores of global identification, of voicing, and of manner and place of articulation scores. For the global score the chance level of success is 8.3%, for voicing it is 50%, for manner of articulation three classes were used and the chance level is 33%, and for place of articulation four classes were used and the chance level is 25%.

RESULTS

As concerns the absolute thresholds of sensitivity, subjects presented very similar values for the various frequencies as well as for both electrodes. This finding is illustrated in Fig 1, which shows thresholds obtained at the basal electrode for the five subjects at different frequencies. For the perception of a gap in noise, durations of silent gap varied among subjects between 55 and 80 milliseconds for the apical electrode and 50 and 90 milliseconds for the basal electrode. For the interval between two impulses, results varied between 2 and 6 milliseconds for the apical electrode and 1 and 6 milliseconds for the basal electrode. There appeared to be no correlation between these two sets of measures of temporal resolution or between results at the apical electrode and those at the basal electrode for each test. For each subject the four scores on the consonant test were measured separately for male and female voices. Correlations between the four measures of temporal resolution and the eight scores on the consonant test were analyzed. Only results of temporal discrimination at the basal electrode for the two-click test indicated some correlation with scores of consonant identification. Since very similar results were obtained for all subjects for the male and female voices, an overall score was computed including both voices. The correlation value for two-click temporal resolution with the global score was −.952 and with place of articulation score it was −.962 — both significant at the 1% level. Figure 2 presents the results obtained on the consonant test for each subject as a function of results for the two-click discrimination at the basal electrode.

DISCUSSION

The results of this study indicate a relationship between patients' speech perception and ability for temporal discrimination in the range of a few milliseconds at the basal electrode, where signals corresponding to high frequencies of speech are delivered. Several previous studies also reported capacities of temporal resolution in this range of a few milliseconds for electrical stimulation of the cochlea similar to those of normal subjects with acoustic stimuli.[9-13] In agreement with the present study, the detection of a gap in noise with electrical stimulation was previously reported to be about one order of magnitude worse than that observed for normal hearing and acoustic stimuli.[11] As observed in our study, two other recent reports indicate that performance on temporal resolution is independent of place of stimulation in the cochlea.[12,13]

It is well known that temporal cues constitute distinctive phonemic features, in particular for consonants, and that acoustic analysis of speech requires a temporal resolution of about 10 milliseconds.[6-8,14] Recent studies on speech perception by patients using cochlear implants gave evidence for the crucial use of fine temporal cues[15] and also stressed the importance of the high-frequency information.[16,17] More specifically, the data from the present study indicating a relation between temporal resolution at a high-frequency channel and identification of place of articulation find some corroboration with an earlier study on one patient reporting the use of abruptness of frequency change as a cue to place identification.[15]

In conclusion, this study indicates that delivering fine temporal coding can be crucial for the success of an implant, and that for a given subject, optimal capacity for temporal resolution may serve as a criterion for choosing a basal electrode.

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NINTH SHAMBAUGH/SHEA WEEKEND OF OTOLOGY

The Ninth Shambaugh/Shea Weekend of Otology will be held March 6-8, 1992, at the Chicago Marriott Downtown in Chicago, Illinois. For further information, contact John J. Shea, MD, 6133 Poplar Pike, Memphis, TN 38119; Phone: (901) 761-9720; Fax: (901) 683-8440.

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PREDICTION OF BEHAVIORAL THRESHOLD AND COMFORT VALUES FOR NUCLEUS 22-CHANNEL IMPLANT PATIENTS FROM ELECTRICAL AUDITORY BRAIN STEM RESPONSE TEST RESULTS

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DONALD W. GOIN, MD
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We have studied the prediction of behavioral threshold and comfort values for 11 patients who use the Nucleus 22-channel cochlear implant by means of the results of electrical auditory brain stem response (EABR) procedures. For prediction purposes, EABR and behavioral testing were done in the same electrode mode configuration for three specific electrodes. The results of this investigation suggest that EABR threshold current level is consistently near the behavioral comfort current level, rather than the patient's behavioral threshold level.

KEY WORDS — cochlear implant, electrical auditory brain stem response.

INTRODUCTION

The electrical auditory brain stem response (EABR) is elicited with electrical rather than acoustic stimuli. The waveform morphology and magnitude of the EABR are similar to those of the acoustic auditory brain stem response (ABR). Our methods and procedures are described in another publication.[1] The absolute latencies of the EABR component waves are shorter than the acoustic responses.[2-6] Typically, wave I of the EABR is absent, because it is usually obscured by stimulus artifact and preamplifier distortion.

We have investigated the prediction of behavioral threshold and comfort (T/C) values for 11 of our adult and child Nucleus 22-channel cochlear implant patients by using the results of EABR procedures. For prediction purposes, EABR and behavioral testing were done in the same electrode mode configuration (common ground, bipolar, bipolar + 1, etc) for three specific electrodes.

METHODS

Subjects. Each of the 11 subjects reported in this paper was evaluated for the Nucleus multichannel cochlear implant according to the appropriate study protocol (adult postlingual, adult prelingual, adult severely hearing impaired, children 2 to 9 years of age, or children 10 to 17 years of age). Eleven patients received EABR testing either intraoperatively at the time of implantation or as outpatients after initial stimulation and programming.

Equipment. The Nucleus cochlear implant receiver-stimulator (CI22M) was implanted in all of the patients. The internal device was controlled by a Nucleus speech processor (WSP III), which was programmed through a Nucleus speech processor interface (SPI). The SPI was connected to a personal computer (IBM portable 5155) using Cochlear Corporation diagnostic and programming software. A TTL trigger pulse corresponding to the digital signal for the phi 2 (second) stimulation phase (positive) from the SPI was used to trigger an evoked potential system (Nicolet Compact-4). The evoked potential system was set for a 10-millisecond analysis time with the electroencephalographic input filtered from 100 to 3,000 Hz for the EABR.

Procedures. The majority of our EABR recordings were obtained at the end of the implant operation, after the surgeon placed the skin flap over the implanted device. Needle insert electrodes were placed on the contralateral mastoid and referenced

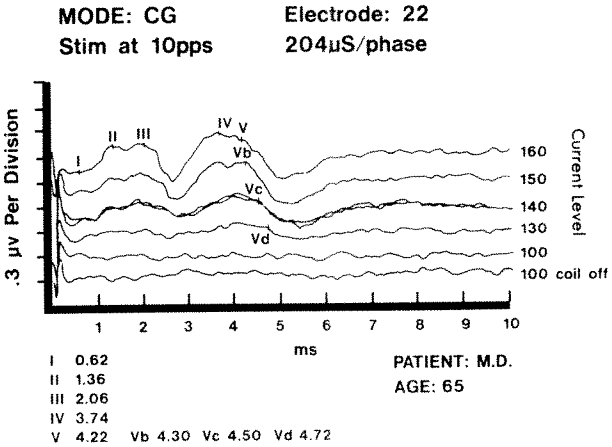

Fig 1. Electrical auditory brain stem response tracings obtained from patient MD correlate well with this patient's behavioral responses to same biphasic electrical pulses presented at 14 pulses per second. His behavioral threshold for this electrode was at current level of 94 and his comfort level was 117. He is patient 1 in Fig 2. CG — common ground, pps — pulses per second.

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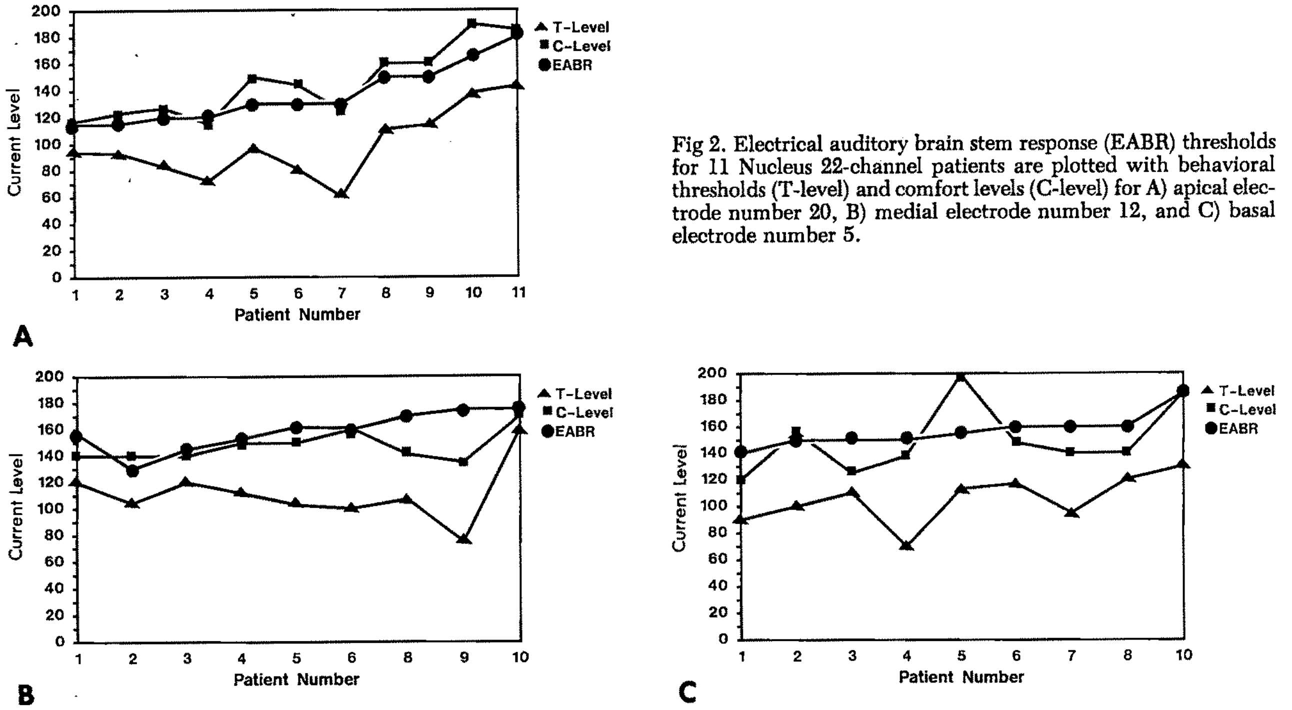

Fig 2. Electrical auditory brain stem response (EABR) thresholds for 11 Nucleus 22-channel patients are plotted with behavioral thresholds (T-level) and comfort levels (C-level) for A) apical electrode number 20, B) medial electrode number 12, and C) basal electrode number 5.

positively to the vertex. A ground electrode was placed on the forehead.

The surgeon positioned a gas-sterilized transmitting coil, including a magnet, over the internal device. The average intraoperative testing time was approximately 10 minutes. Testing occurred while the surgeon sutured the skin flap, thus minimizing the need to extend the time that the patient was under general anesthesia.

Patients were tested for behavioral T/C measurements approximately 1 month after the implant operation. They were seated comfortably in a chair, and the transmitting coil with a centering magnet was placed over the internal device as described above. The patients were instructed to report "first hearing" of the electrical stimuli (threshold) and maximum comfort level for each of the electrodes.

Stimuli. Biphasic current pulses (205 microseconds per phase) were presented at a rate of 10 to 14 pulses per second. The stimulation mode was typically common ground or bipolar plus 1 at a pulse width of 205 microseconds per phase. All EABR measurements were obtained in current level. An apical electrode (E22 or E20) was tested first, followed by a medial (E12) and/or basal electrode (E5). The nominal stimulation current was set initially at a level of 80 to 100 and increased by 20 levels until an identifiable EABR was observed. Then, additional recordings were collected in sequential increments (5 to 10 levels) up to 30 to 40 current levels above the first consistent response. Typically, 400 to 600 averages were adequate for each intraoperative EABR recording.

Measurements. We measured the EABR wave amplitudes and latencies as a function of the current levels. The EABR threshold was the current level at which the last discernible response was noted. We also measured the behavioral T/C levels in the current level. The behavioral T/C values were the average of at least three trials using either the method of adjustment or the method of limits.

RESULTS

Typical EABR tracings from an adult patient (MD, age 65) are shown in Fig 1. With the Nucleus multichannel implant, it is possible to stimulate various portions of the cochlea (eg, basal versus apical) independently. For patient MD, the electrode is apical (E22). In comparison, his threshold and comfort levels in the common ground mode at a pulse rate of 200/s were T = 94 and C = 117. The EABR waveform morphology demonstrated the presence of waves II through V as typically observed in the ABR. However, the absolute latencies for the EABR were shorter than typical ABR values. Also, wave I is absent because the EABR recording electrodes were placed on the contralateral side of the head from the cochlear implant. Ipsilateral EABR recordings typically have a wave I that is obscured by stimulus artifact.

To study the relationship between EABR and the behavioral measurements of T and C levels, we rank-ordered the EABR current level thresholds for the 11 patients. These values were then plotted with the T/C values versus current level. The relationships between EABR threshold and the behavioral T/C values for 11 patients are shown in Fig 2. The values for each electrode demonstrate our main finding that EABR threshold current level is consistently near the behavioral comfort (C) current level rather than the patient's behavioral threshold (T) level. Patient MD (Fig 1) is patient 1 in Fig 2, and one can note the close agreement between his EABR threshold and his measured behavioral C level response.

SUMMARY

There are several possible applications for the EABR with cochlear implant patients. We can objectively assess device function or failure at the time of operation or when device functioning is questionable. Neural survival within the various segments of the cochlea can be assessed. The potential for an objective measure for loudness growth in the setting of the speech processor is evident from our results. However, it is apparent in our findings that the EABR threshold correlated better with behavioral C levels than with T levels in our evaluation of 11 patients.

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FIRST INTERNATIONAL CONFERENCE ON ELECTROCOCHLEOGRAPHY AND MONITORING

The First International Conference on Electrocochleography and Monitoring will be held Sept 20-24, 1992, in Wurzburg, Germany. For further information, contact Program Chairman, Dr Dirk Hoehmann, Dept of Otolaryngology, University of Wurzburg Medical School, Josef-Schneider-Str 11, D-8700, Wurzburg, Germany; telephone 49-931-201-2323, or FAX 49-931-201-2248.

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LARYNGEAL MANIFESTATIONS OF GOUT

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Gout is a disorder of purine metabolism characterized by hyperuricemia with rare involvement of the head and neck. We present a 72-year-old woman with a known history of gout who presented with hoarseness and a lesion suspicious for carcinoma of the larynx. Endoscopic biopsy revealed a tophus of the true vocal cord with characteristic birefringent crystalline deposits and giant cell granuloma. There have been limited reports of gouty involvement of the larynx, more commonly involving cricoarytenoid arthritis. Tophi of the laryngeal soft tissues are exceedingly rare. In this paper we will discuss the pathophysiology and management of this interesting clinical entity.

KEY WORDS — gout, larynx.

INTRODUCTION

Gout is a disease that the otolaryngologist rarely encounters; therefore, the pathophysiology, diagnosis, and management of gout may be unfamiliar. However, this disease can have significant involvement of head and neck sites. Although the larynx is an infrequently affected site, gouty involvement of the larynx can lead to significant clinical disease.

The prevalence of gout in the United States is estimated at 0.2%.[1] It is more common in men, with 95% of cases occurring in men, with a peak incidence in the fifth decade.[2] Hyperuricemia is present in 2% to 18% of the population.[3] Approximately 5% of hyperuricemic patients develop the clinical signs and symptoms of gout.

Acute gouty arthritis is an exquisitely painful, monoarticular arthritis, most commonly affecting the first metatarsophalangeal joint. Following the acute gouty arthritis is an asymptomatic period termed the intercritical period, followed by intermittent gouty attacks. Subsequent attacks of gouty arthritis may be polyarticular. The occurrence of tophi, or chronic tophaceous gout, is a late sequela of long-standing hyperuricemia. The diagnosis of gout is typically made by microscopic examination of synovial fluid with the demonstration of negatively birefringent urate crystals and an inflammatory response typical of acute gouty arthritis.

A case report of chronic tophaceous gout of the larynx is presented with a discussion of the pathophysiology of the laryngeal manifestations of gout and a review of the literature.

CASE REPORT

A 71-year-old woman presented with a chief complaint of increasing hoarseness over a period of 1 month. She had mild dysphagia and throat pain without dyspnea. A 50-pack year history of smoking was noted, as well as occasional ethanol use. Her past history was significant for diabetes, hypertension, and a long history of gout, most significantly in the joints of the hands and feet. Physical examination revealed an exophytic mass of the right true vocal cord, with decreased mobility. The neck was normal to palpation. She had a gouty tophus of the left great toe. Plasma uric acid was 10.1 mg/dL. A barium swallow study yielded normal findings. Computed tomography of the head and neck revealed an enhancing lesion of the right endolarynx and soft tissue opacification lateral to the right laryngeal cartilages consistent with neoplasm or inflammation. The patient was brought to the operating room, where a lesion involving the right false vocal cord, laryngeal ventricle, and right true vocal cord was submitted to biopsy.

Formalin-fixed, hematoxylin and eosin-stained sections of laryngeal tissue and true vocal cord (see Figure, A) revealed hyperplastic squamous mucosa without malignancy. The submucosa was diffusely infiltrated by numerous microtophi, surrounded by a prominent foreign body giant cell reaction (see Figure, B), admixed with lymphocytes and histiocytes. The microtophi consisted of abundant amorphous deposits of negatively birefringent material (see Figure, C). Examination of the tophi with use of a primary color compensator lens confirmed the urate origin of the birefringent deposit. When the compensator lens was positioned between two polarizing lenses, crystals were confirmed to be of monosodium urate composition, as they changed from yellow to blue upon rotation of the polarizing

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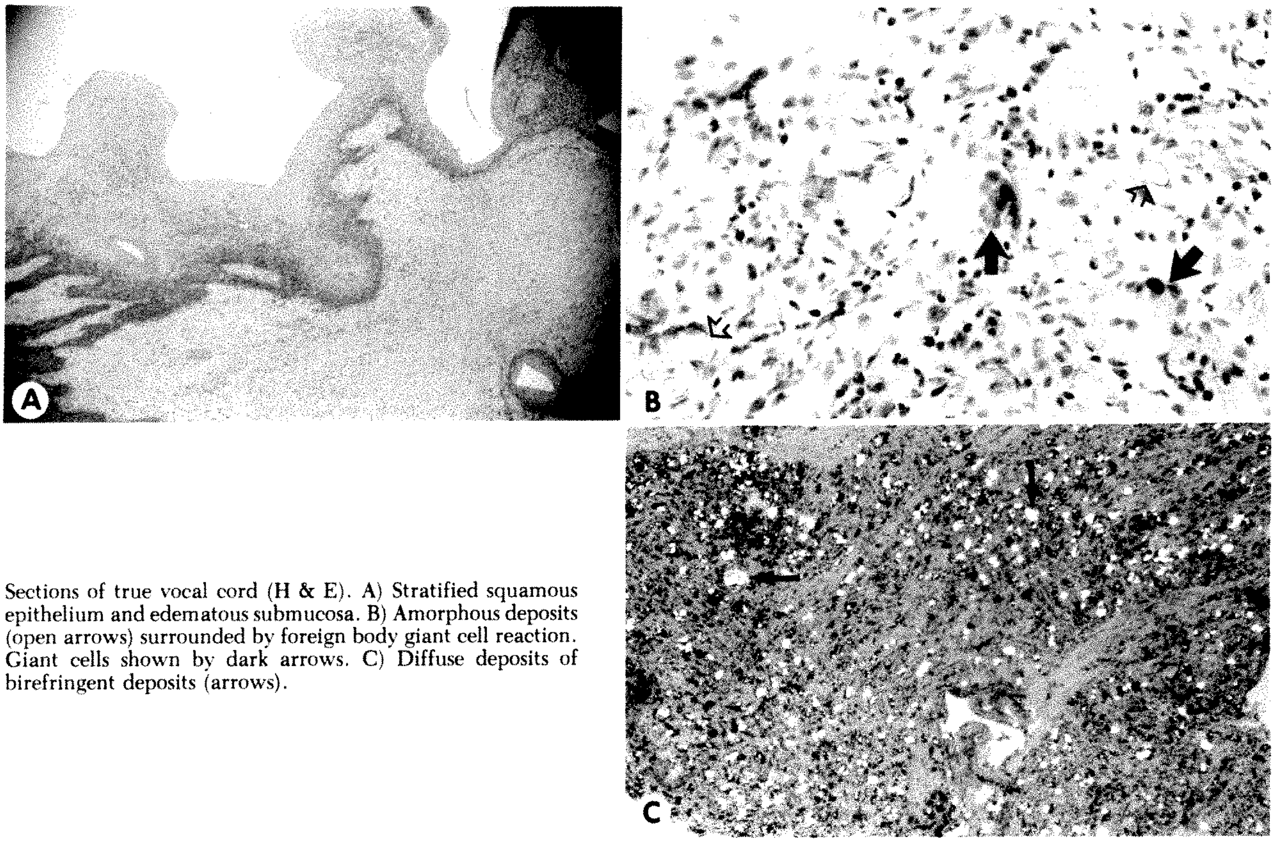

Sections of true vocal cord (H & E). A) Stratified squamous epithelium and edematous submucosa. B) Amorphous deposits (open arrows) surrounded by foreign body giant cell reaction. Giant cells shown by dark arrows. C) Diffuse deposits of birefringent deposits (arrows).

lenses, from parallel to perpendicular, respectively.

The patient had an uneventful postoperative course with some resolution of laryngeal symptoms at 2 weeks. Laryngeal examination showed marked resolution of the mucosal abnormalities but continued paresis of the right true vocal cord. The patient was referred to the rheumatology service for consultation.

DISCUSSION

Gout has a long history and often has been associated with royalty. Hippocrates wrote about this disease in the fifth century BC. The first description of a gouty patient with involvement of the larynx was made by Garrod[4] in 1863. In his review of the problem of gouty laryngitis, Ewart[5] listed symptoms of hoarseness, cough, and scanty and occasionally blood-stained expectoration. Although gout is a common affliction, its pathophysiology and treatment have only become clearly understood in the past 30 to 40 years. As this case report demonstrates, gout can significantly affect the larynx.

Gout may affect the head and neck in any stage of the disease. The initial attack of gouty arthritis rarely affects head and neck joints. In chronic gouty arthritis, any joint, including the sternoclavicular joint, temporomandibular joint, or cricoarytenoid joint, may be affected.

Gouty cricoarytenoid arthritis has had few case reports, as summarized in Table 1[6-8]. Typically, the patient with involvement of the cricoarytenoid joint has had long-standing chronic polyarticular arthritis. Symptoms include pain, dysphagia, and hoarseness. Cricoarytenoid fixation can be demonstrated on direct laryngoscopy.

Laryngeal symptoms during colchicine therapy for gouty arthritis of nonlaryngeal sites were reported by Mournier-Kuhn and Wild,[6] Montgomery and Lofgren,[8] and Montgomery.[9] However, in 1976, Goodman et al[7] described the first case report with pathologic confirmation. In their patient, who had marked laryngeal symptoms for 1 year and bilaterally fixed vocal cords, autopsy revealed cricoarytenoid arthritis as well as involvement of the spine and sternoclavicular joints. This patient also had tophi in the ear, tongue, hypopharynx, and subglottic larynx.

Tophi have more frequently been reported in the head and neck, while reports of tophaceous involvement of the larynx are still few (Table 2[1,3,4,7,10-17]). Garrod[4] described specks of urate on the arytenoid cartilage of a patient with severe tophaceous gout. Virchow[10] described pinhead-size grains of urates on the vocal cords of a gouty man in 1868. Litten[11] reported a patient with generalized gout with tophi in palatine tonsils, soft palate, uvula, hyoid, and glossal-epiglottic ligaments. Moore[12] noted urate deposits lodged in both vocal cords. Jackson and Jackson[16] reported a case of a man with a long

TABLE 1. CASE REPORTS OF GOUTY CRICOARYTENOID ARTHRITIS

Author	*Description*
Montgomery and Lofgren[8]	R true vocal cord paralysis, resolution on colchicine
Goodman et al[7]	1-year history of symptoms, arytenoid fixation, postmortem diagnosis
Mournier-Kuhn and Wild[6]	History of irradiation, required tracheotomy, resolution on colchicine

history of tophaceous gout who presented with a lesion and fixation of the right arytenoid and true vocal cord. Jackson and Jackson were suspicious of carcinoma; however, biopsy revealed laryngeal tophi. Okada[13] reported a patient with tophi of the laryngeal ventricle, and Lefkovits[3] reported true vocal cord tophi. Ferlito and Bonito[17] reported a postmortem finding of a pea-size tophus of the right subglottis. Marion et al[14] reported tophi of the right true vocal cord. Finally, Goodman et al[7] reported laryngeal tophi in one patient. A total of eight patients are found in the world literature with reports of laryngeal tophi. Reports of other head and neck sites with tophaceous gout include the tongue, the temporomandibular joint (with extension to the eustachian tube and nasal cartilages), and, most commonly, the pinna.[1,15] As in our patient and in the report of Jackson and Jackson, tophi of the true vocal cords can easily be confused with malignancy. A computed tomographic scan may not be helpful in differentiating these two processes.

With the advent of modern treatments for gout, the frequency of tophaceous gout has decreased. Now less than 17% of patients with gout develop tophi.[1] The decreased awareness by clinicians of gouty laryngeal involvement may relate to this overall decrease of tophaceous gout.

Although painless, extensive tophi can result in significant symptoms and morbidity when involving the head and neck. The management of gout not only involves treatment of acute gouty arthritis, but one must also prevent recurrences and treat associated factors such as obesity, hypertension, and hyperlipemia, and prevent and treat renal disease. Colchicine is the cornerstone of treatment of acute gouty arthritis. Its mechanism of action is not clearly understood. Indomethacin and other nonsteroidal anti-inflammatory agents are also efficacious in the treatment of acute gouty arthritis. Colchicine can be used in small daily dosages as prophylaxis for gouty arthritis, but this will not prevent the tophaceous effects of hyperuricemia. In contrast, treatment of hyperuricemia will usually prevent the tophaceous effects of gout. The goal of antihyperuricemia therapy is to maintain uric acid at less than 7 mg/dL. Allopurinol is the most commonly used agent in the treatment of hyperuricemia. Adjuvants to medical therapy include diet, weight reduction, and decreased ethanol consumption. Kelly and Fox[2] noted, “Since the advent of effective antihyperuricemic therapy, only a minority of patients develop visible tophi, permanent joint changes or chronic symptoms; thus, effective therapy alters the natural history of this disease.”

TABLE 2. CASE REPORTS OF HEAD AND NECK TOPHI

Author	*Year*	*Location*
Garrod[4]	1863	Arytenoid cartilage*
Virchow[10]	1868	Larynx*
Litten[11]	1876	Oral cavity, hyoid
Moore[12]	1882	R and L true vocal cords*
Jackson and Jackson[16]	1942	True vocal cord*
Okada[13]	1964	Laryngeal ventricle*
Lefkovits[3]	1965	True vocal cord*
Marion et al[14]	1972	R true vocal cord*
Ferlito and Bonito[17]	1974	Subglottic
Goodman et al[7]	1976	Tongue, larynx*
Hyams†	1981	Nasal septum
Faas[15]	1983	Temporomandibular joint, eustachian tube

*Laryngeal tophi.

†Personal communication mentioned by Stark and Hirokawa.[1]

Laryngeal tophi represent a special entity and may require surgical intervention. It can be a diagnostic problem requiring biopsy to rule out malignancy. While medical therapy is efficacious in the treatment of tophaceous gout at extralaryngeal sites, Okada[13] states that medical therapy is of little value in treating hoarseness of tophaceous involvement of the larynx. Both Lefkovits[3] and Marion et al[14] demonstrated the utility of endoscopic removal of laryngeal tophi, with resolution of symptoms and follow-up examination demonstrating no recurrence. Medical therapy can be efficacious in the treatment of acute gouty arthritis of the cricoarytenoid joint.

The small number of case reports in the literature may reflect the limited awareness of gouty laryngopathy among clinicians and not reflect the true incidence of this entity. Lefkovits[3(p1023)] states,

> It appears likely that deposition of urate crystals in the larynx in the vicinity of the vocal cords occurs, and not too infrequently. Because such deposition in most cases excites only a mild reaction locally and induces minor symptoms — namely insidious development of hoarseness or slight sore throat, both the doctor and patient pay little attention to it; laryngoscopic examination is therefore not done and the condition is not recognized. Only when sufficiently severe symptoms develop as a consequence of uric deposition in the larynx, is the latter examined and the condition discovered.

SUMMARY

A case report is presented of a 71-year-old woman with tophi of the true vocal cord. This is the ninth report of a laryngeal tophus in the literature and the only report in a woman. That laryngeal involvement with either tophaceous or gouty arthritis is so rarely reported in the literature may not be solely a reflection of its uncommon involvement of that site.

There may be an element of misdiagnosis in these patients. The threshold of suspicion for gouty laryngopathy and the awareness of this clinical entity is generally low among clinicians. Increased awareness of this disorder may lead to increased clinical recognition. The patient with known gout and symptoms related to the larynx should be evaluated by an otolaryngologist. Lower thresholds of suspicion will allow earlier diagnosis and treatment of this interesting malady.

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7TH WORLD CONGRESSES FOR BRONCHOLOGY & BRONCHOESOPHAGOLOGY

The 7th World Congresses for Bronchology & Bronchoesophagology will be held Sept 28-Oct 2, 1992, at the Mayo Clinic and Mayo Medical Center in Rochester, Minnesota. For further information, contact Udaya B. S. Prakash, MD, Secretary-General & Director, 7th WCB & WCBE, East-18, Mayo Clinic, Rochester, MN 55905.

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CAUSES OF CHILDHOOD DEAFNESS AT A DUTCH SCHOOL FOR THE HEARING IMPAIRED

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This study was conducted on 162 hearing-impaired school pupils who were investigated as to the cause of their hearing loss. In 64 pupils (40%) a hereditary cause was recognized, and in 43 (27%), an acquired cause; in 55 (34%) the cause remained unknown, according to the criteria we used for defining a cause. Special attention was paid to the differences between the hereditary and acquired forms of deafness in relation to the degree of hearing loss. The study population therefore was divided into groups per 10 dB of hearing loss. Using this method we found that an autosomal dominant hearing loss occurred significantly more frequently among the less severe hearing disorders. This finding, which has not been seen in the literature, is important in genetic counseling for the deaf. Another remarkable finding was that Usher's syndrome, especially type 2 with a moderate hearing loss, was more frequent among the hearing-impaired subjects than we had expected.

KEY WORDS — acquired deafness, etiology, genetic counseling, genetic deafness, hearing impairment, Pendred's syndrome, Usher's syndrome.

INTRODUCTION

When studies on the causes of hearing impairment and deafness were investigated, the results were roughly equally divided over three main categories: hereditary, acquired, and unknown (Table 1[1-8]). Comparison of these studies[1-8] showed considerable differences in the composition of the populations studied, as well as in the way the investigations had been carried out. Owing to the absence of generally accepted criteria defining a cause as such, the researcher has a great deal of influence on his results. This influence was minimized in the studies carried out in Manchester,[4,8] Copenhagen,[5,7] Nijmegen,[3] and this study, because they were carried out with the senior researcher in that location.

Most studies reported subjects with different degrees of hearing loss, but no attention was paid to a possible relationship between the degree of hearing impairment and its cause. Although the transition from hearing impairment to tone-audiometric deafness is gradual, it is nevertheless the case that around 90 dB the power of discrimination and therefore speech comprehension decreases to such an extent that this may be used as the bottom line for deafness.[9]

The main studies included subjects with all degrees of hearing loss; only Cremers[3] described a population comprising solely deaf people, with a hearing loss of more than 90 dB Fletcher index. This study showed a remarkably low percentage of autosomal dominant mode of inheritance, contrary to the other studies.

TABLE 1. CAUSES OF EARLY CHILDHOOD HEARING IMPAIRMENT AND DEAFNESS (FROM EIGHT STUDIES)

Cause	*Fraser*[1] *(n = 2,355; hearing impaired or deaf)*	*Fraser*[2] *(n = 3,535; hearing impaired or deaf)*	*Cremers*[3] *(n = 60; deaf)*	*Newton*[4] *(n = 111; hearing loss >25 dB)*	*Holten and Parving*[5] *(n = 94; hearing loss >65 dB)*	*Kankkunen*[6] *(n = 179; hearing loss >25 dB)*	*Parving*[7] *(n = 117; hearing loss >35 dB)*	*Taylor et al*[8] *(n = 86; hearing impaired or deaf)*
Hereditary	32	49.6	37	25	33	55	48	24
Autosomal dominant	ID	15.2	2	12	ID	ID	ID	ID
Autosomal recessive	ID	32.7	35	13	ID	ID	ID	ID
X-linked	ID	1.7	ND	ND	ID	ID	ID	ID
Acquired	32	50.4	38	32	41	29	42	48
Meningitis	17	ID	13	4	7	7	3	6
Rubella	6	ID	5	11	15	5	19	8
Kernicterus	4	ID	15	14	15	1	14	19
Asphyxia	1	ID	2			1		15
Otitis	1	ID	ND	ND	1	6	3	ND
Others	3	ID	3	2	3	9	3	ND
Unknown	36	ND	25	43	26	16	10	28

Data are percentages.
ID — insufficient data, ND — no data.

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REPRINTS — Peter M. van Rijn, MD, PhD, Dept of Otorhinolaryngology, University Hospital Dijkzigt, Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands.

During the past two decades of dealing with genetic counseling for the hearing impaired, we have observed that an autosomal dominant mode of inheritance appears more frequently among the less severe hearing losses. We therefore planned to investigate a population of mainly hearing-impaired subjects for their causes of hearing loss, with special attention to the genetic hearing disorders.

PATIENTS AND METHODS

The study population comprised pupils and ex-pupils of a school for the hearing impaired in Nijmegen, the Netherlands, born between January 1, 1960, and December 31, 1975, with an average hearing loss of 35 dB or more in the better ear (Fletcher index). Of the 174 subjects who met these selection criteria, 162 participated in our study. Two subjects, 1 operated on for congenital stapes ankylosis and 1 for chronic otitis media, were excluded from this study because their postoperative hearing loss was less than 35 dB. The average age of the subjects was 20 years. The study group of 162 comprised 91 males (56%) and 71 females (44%). The average hearing loss of the total population was 62 dB, ranging from 35 dB to only a few subjects with more than 120 dB.

All the subjects and their families were visited at home and additional diagnostic procedures, as described below, were subsequently performed at the Nijmegen University Hospital on all the subjects.

A general physical examination was performed in order to detect or verify syndromes that might not have been perceptible at the toddler age.

All the 162 subjects and both parents (if still alive) were examined audiometrically. A standard pure tone audiogram with air and bone conduction was performed for the octave frequencies 250 to 8,000 Hz. When indicated, the half-octave frequencies were also measured in order to detect a possible dip in the audiogram as seen in some carriers of autosomal recessive deafness.[10]

Every subject was seen by an ophthalmologist and was subjected to a general ophthalmologic examination, including fundoscopy in mydriasis. If pigment defects were found that might have matched a viral embryopathy or matched the description of Usher's syndrome, an electro-oculogram, an electroretinogram, and fundus pictures were taken. If tapetoretinal degeneration was suspected, visual field investigations and adaptation to darkness were also carried out.[11] Vestibular examinations consisted of measuring the vestibulo-ocular reaction after caloric stimulation and the velocity-step test.[12]

A genealogic investigation was carried out in order to detect consanguinity between the parents. We routinely searched up to five generations back. If suspicion arose about consanguinity, the investigation was extended.

All the pupils were tested for congenital syphilis (*Treponema pallidum* hemagglutination assay and Wasserman tests) and thyroglobulin and thyroid-stimulating hormone levels, in order to detect Pendred's syndrome in an alternative way. Recently, in some patients with partial organification defects of the thyroid, as are also found in Pendred's syndrome, extremely high thyroglobulin levels were found.[13] We therefore thought that it would be useful to screen our study population for their thyroglobulin levels, in order to avoid using the radioactive materials of the perchlorate test.

After collecting all the data described above and receiving the necessary information from other medical colleagues, we wrote a report on every subject. The results and conclusions were then discussed with a pediatrician, an obstetrician, a geneticist, an ophthalmologist, and an otorhinolaryngologist. Written criteria were used for the acceptance of hereditary and acquired causes.[14]

An autosomal recessive form of inheritance was accepted if at least one of the following criteria was present and if there were no indications for another cause.

1. At least two hearing-impaired children in one family without any other apparent cause for this disability.
2. Proven consanguinity between the parents within five generations.
3. Early hearing impairment in at least two others of the subject's relatives.
4. An apparent autosomal recessive form of hearing impairment in the subject's relatives.
5. Autosomal recessive inherited syndromes.

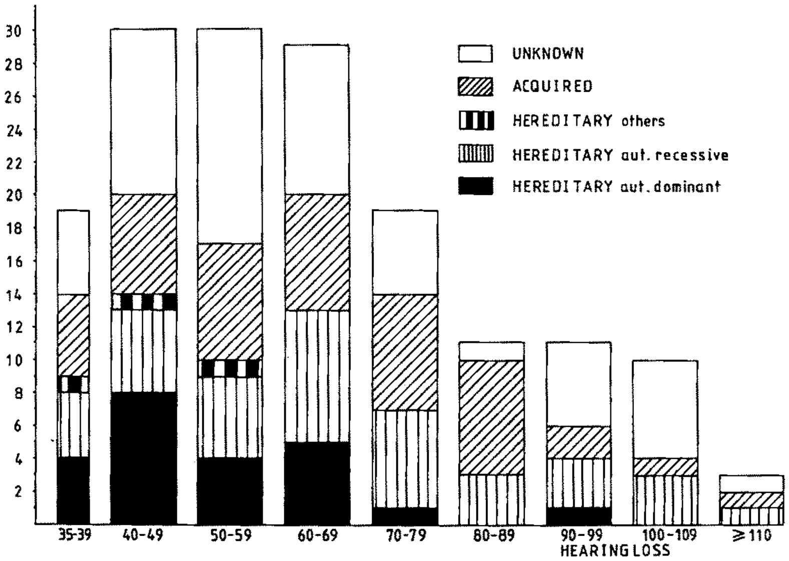
To accept an autosomal dominant mode of inheritance, at least one of the following criteria had to be present.

1. Hearing impairment in at least three successive generations, according to history.
2. Audiometrically proven hearing loss in at least two generations.
3. Autosomal dominant inherited syndromes.

If these criteria were not met, or if more than one cause was possible, the cause was considered unknown.

RESULTS

In our study a definite cause for the hearing loss could be found in 107 of the 162 pupils investigated (66%), in accordance with our criteria. In 55 (34%) the cause remained unknown despite thorough examination. In 64 pupils (40%) a hereditary cause was recognized, and in 43 (27%) an acquired cause. Hereditary hearing disorders are usually divided into syndromal and nonsyndromal hearing losses. Our hereditary group exhibited mainly syn-



Causes of hearing loss in 162 students from school for hearing impaired, divided into bands of 10 dB according to their audiograms.

dromal characteristics (14 of 162 or 9%). Apart from the hereditary and acquired causes, we also distinguished a group in which an audiometrically recognizable type of hearing loss was present, eg, low-, middle-, or high-frequency sensorineural hearing loss.

In the present study, the causes were divided, according to the degree of hearing loss, into bands of 10 dB (see Figure). In this way, a relationship was investigated between the cause and the degree of the hearing loss.

Using this method we found that subjects with an autosomal dominant inherited hearing disorder (23 or 14%) were found significantly more frequently among the less severe hearing losses (<70 dB; $p<.05$; see Figure). This diagnosis could be recognized in the less severely hearing-impaired subjects by the routine audiometric examination of all the parents. Some of the parents and other relatives were not even aware of their hearing loss. In the autosomal dominant group, four subjects were found to have a syndrome: dysostosis mandibulofacialis was found twice, one subject had the branchio-oto-renal syndrome, and another one was known to have Waardenburg's syndrome (Table 2).

Of the seven audiometrically recognizable types of autosomal dominant hearing loss, six subjects were found to have middle-frequency deafness. In three of them the hearing loss was progressive. In one, a high-frequency hearing loss was found. It is possible that on the grounds of the selection criteria (hearing loss of more than 35 dB on the "speech frequencies" 500 to 2,000 Hz) this form of hearing loss is occasionally missed. Half of the subjects with autosomal dominant hearing losses (7%) did not have any other abnormalities.

The autosomal recessive hearing disorders, diagnosed in 38 subjects (23%), were found relatively more frequently among the severe hearing losses. This finding was not significant in our study (see Figure). Usher's syndrome type 2[15] was diagnosed in 5 subjects (3%); only 1 of them was previously aware of the diagnosis. The average hearing loss of all 5 subjects was 55 dB. All these subjects had passed adolescence when the diagnosis was made. Two other autosomal recessive syndromes were found: one case of pili torti syndrome[16] and one case of renal tubular acidosis syndrome.[17]

TABLE 2. HEREDITARY CAUSES OF HEARING IMPAIRMENT IN 64 OF 162 PUPILS AT SCHOOL FOR HEARING IMPAIRED

		Subjects	
		No.	*%*
Autosomal recessive forms of hearing loss			
Syndromal		7	4
Usher's syndrome	5		
Pili torti and sensorineural deafness	1		
Renal tubular acidosis and sensorineural deafness	1		
Audiometrically recognizable type of hearing loss		5	3
Progressive high-frequency deafness	4		
Low-frequency deafness	1		
Nonspecific hearing loss		26	16
Autosomal dominant forms of hearing loss			
Syndromes		4	3
Dysostosis mandibulofacialis	2		
Branchio-oto-renal syndrome	1		
Waardenburg's syndrome	1		
Audiometrically recognizable type of hearing loss		7	4
Progressive middle-frequency deafness	6		
High-frequency deafness	1		
Nonspecific sensorineural hearing loss		12	7
Others		3	2
Klippel-Feil syndrome	1		
Alport's syndrome	1		
X-linked mixed deafness syndrome with perilymphatic stapes gusher	1		
Total		64	40

TABLE 3. CAUSES OF HEARING IMPAIRMENT IN 162 PUPILS AT SCHOOL FOR HEARING IMPAIRED

	No.			*%*	
Hereditary			64		40
Autosomal recessive		38		23	
Syndromal	7				
Audiometrically recognizable type	5				
Nonspecific	26				
Autosomal dominant		23		14	
Syndromal	4				
Audiometrically recognizable type	7				
Nonspecific	12				
Other pattern of inheritance		3		2	
X-linked	1				
Unclear	2				
Acquired			43		27
Prenatal		11		7	
Rubella	9				
Teratogenic medication	2				
Perinatal		14		9	
Kernicterus	10				
Other perinatal causes	3				
Meningitis	1				
Postnatal		18		11	
Meningitis	8				
Chronic otitis media	6				
Ototoxic medication	3				
Measles	1				
Unknown			55		34
Evidence of more than one cause		8		5	
Insufficient evidence for a cause		20		12	
No evidence at all		27		17	
Total			162		100

Percentages are rounded.

Audiometrically recognizable types of autosomal recessive deafness were found in four subjects with progressive high-frequency deafness[18] and in one subject with low-frequency deafness. The latter type has not been previously described in the literature. The majority of hearing-impaired subjects with an autosomal recessive pattern of inheritance (26%) showed no other characteristics besides their hearing loss.

In one subject, the mode of inheritance was heterosomal.[19] Although a syndrome could be recognized in two subjects, namely Alport's and Klippel-Feil syndromes, the pattern of inheritance remained unclear. In Klippel-Feil syndrome the genetic background is doubtful.

The acquired forms of hearing impairment (27%) were divided into prenatal (7%), perinatal (9%), and postnatal (11%) causes (Table 3).

In our study, nine (6%) cases were caused by congenital rubella, eight of which showed characteristic pigment abnormalities. For two of these subjects, the diagnosis could only be made on the basis of the ophthalmologic findings. In all nine subjects, visual function was normal. The history and ophthalmologic examination were the most valuable means for finding this cause. It was remarkable that prenatal laboratory tests for rubella had only been carried out in one affected child. In view of the retrospective nature of the present study, the diagnosis of congenital cytomegalovirus infection could not be demonstrated, because the necessary serologic investigations had not been carried out in the prenatal or perinatal period. During the prenatal period, two subjects had received teratogenic medication: quinine and thalidomide,[20] respectively.

Kernicterus (10 of 162 or 6%) in the perinatal period was assumed to be the most important cause of hearing loss. Five of these subjects showed a characteristic downward-sloping audiogram.[21] Hypoxia was diagnosed in only 3 subjects (3 of 162 or 2%), who suffered a severely complicated perinatal period, including hypoxic periods. In one of them, ototoxic medication was also prescribed in the perinatal period.

None of the pupils with kernicterus or hypoxia in our study showed severely disturbed psychomotor development. Multihandicapped pupils were educated at other specialized schools in the Netherlands and were therefore expected to be excluded from our study by the selection criteria.

In the postnatal period, meningitis was the most frequently recognized cause (8 of 162 or 5%). *Neisseria meningitidis* (4 cases) and *Streptococcus pneumoniae* (2 cases) were the most frequently cultivated microorganisms. There was one subject with *Escherichia coli* and another with echovirus; in one subject the organism could not be determined because of previously given antibiotics. Despite the ototoxic antibiotics prescribed for three of these children, we attributed their hearing loss to meningitis. Chronic otitis media was found in six subjects (4%), three of them with cholesteatoma.

The group of 55 subjects (34%) with an unknown cause of hearing loss included 28 (17%) in whom one or more indications for a cause were found. However, they did not meet our criteria and their causes of hearing loss were therefore regarded as unknown. In 27 subjects (17%) no signs at all could be found. We could not find a relationship between any of the acquired forms of hearing disorders or unknown causes and the degree of hearing loss (see Figure).

The whole vestibular examination with caloric stimulation and the velocity step test was carried out in 121 of the 162 subjects (75%), without any selection. The distribution of the causes in the vestibular examination group was comparable to that in the total group of 162 subjects. In 43 subjects (36%) the vestibular function was normal, in 28 (23%) hyperreactive, and in 50 (41%) hyporeactive. Decreased vestibular function was found in an equal proportion among the categories hereditary, acquired, and unknown. Hyporeactive functions were found significantly more frequently among the subjects with a hearing loss above 90 dB. The hyporeactive group included 4 subjects with vestibular areflexia. In the acquired group, hyporeactive vestibular function was mainly found among the meningitic and rubella causes. The results of the vestibular examinations in this study did not contribute to the detection of the cause of the hearing loss.

The male predominance in our study population (91 males [56%] and 71 females [44%]) has also been reported in other studies[2,22,23]; a convincing explanation is still lacking.

DISCUSSION

In the past, several studies on hearing-impaired and deaf people were performed to find the causes of deafness. All these studies were retrospective in character. Unfortunately, a great deal of valuable information is lost and some tests are useless after the perinatal period, eg, laboratory tests for cytomegalovirus infection and rubella. Therefore, many questions remain unanswered, especially in cases in which a hearing-impaired subject wishes to receive information about his or her handicap.

In previous studies, little attention was paid to the degree of hearing loss in relation to its cause. In a previous Dutch study that dealt only with severely deaf people, an unexpectedly low percentage of autosomal dominant inherited hearing disorders was found.[3] Reviewing other previous studies, we find that most of them included subjects with both slight and severe hearing disorders.

This study, on students from a school for the hearing impaired, focuses on the cause of sensorineural childhood hearing loss. Special attention was paid to the differences between the autosomal dominant and autosomal recessive forms of deafness in relation to the degree of hearing loss. The study population was therefore divided into groups according to the degree of hearing loss, per 10 dB.

Using this method, we found that an autosomal dominant hearing loss was significantly more frequent among the less severe hearing disorders. An autosomal dominant cause was found in 21 of the 116 subjects with a hearing loss of between 35 and 70 dB Fletcher index, as opposed to 2 in the group of 46 subjects with an average hearing loss of more than 70 dB ($\chi^2 = 4.05$ and $p < .05$). This finding has not been previously reported in the literature. The routine audiometric examination of both parents was an indispensable means for finding autosomal dominant hearing disorders. Some of these parents were not even aware of their hearing loss.

The results of the parents' audiograms seemed to form an important clue in the diagnosis of autosomal dominant inheritance; we could not find any abnormalities in the audiograms of obligate carriers of an autosomal recessive gene for deafness.[10] In another, recent study, audiometric abnormalities of obligate carriers of an autosomal recessive gene could likewise not be found.[24]

The percentage of autosomal recessive hearing disorders in our study (23%) was low, especially in comparison with other studies. We accepted an autosomal recessive form of inheritance only if the previously mentioned criteria were met. In 17 subjects, only scanty evidence for an autosomal recessive mode of inheritance was found. In 8 of them, there were also other indications for an acquired cause (1 rubella contact and 7 with perinatal problems). In the remaining 9, the evidence was insufficient according to the criteria. Therefore, the cause of hearing disorder in these 17 subjects was considered to be unknown.

Consequently, the group with an unknown cause for their hearing disorder was large (34%). We chose this approach in order to be more accurate regarding the percentage of subjects with a particular cause.

Usher's syndrome was found more frequently in the group of subjects with mainly less severe hearing disorders than would have been expected from the literature. Type 1, with profound deafness, is said to be more prevalent than type 2. In our study, five subjects (3%) were found with type 2; in four this came as an unexpected diagnosis. The lower percentage of this syndrome in previous studies could be explained by the fact that the participants were examined at a younger age, when the typical ophthalmologic features could have been missed. In view of the high frequency of Usher's syndrome among the less severe hearing disorders, it seems important to examine these children before adolescence.

Pendred's syndrome, for which a frequency of 5% is reported in the literature, could not be detected in our study. It is not clear whether Pendred's syndrome was missed by us or whether it occurs less frequently among the less severe hearing disorders. We used an alternative screening method for the detection of this syndrome: thyroglobulin and thyroid-stimulating hormone levels. However, the reliability of this cheap and simple screening method should be tested on untreated Pendred's syndrome patients before conclusions are drawn.

In 55 subjects (34%) the cause remained unknown. Owing to the absence of generally accepted criteria on the basis of which researchers may accept a cause, the researcher can have a great deal of influence on the results. Every keen researcher likes to see results and will possibly be inclined to avoid labeling a group as "unknown."[2,7]

In our study, we used criteria that had previously been laid down to draw our conclusions about the hereditary and acquired causes.[14] It is possible to state that in the group of subjects with an unknown cause for their hearing loss, an autosomal recessive mode of inheritance is a frequent cause.[25]

In our study, if subjects were thought to have more than one possible cause of hearing loss, the cause was considered to be unknown. Similarly, in half of the group with an unknown cause (17%), we found indications for one or more causes, but these findings did not meet our criteria.

It would be useful to compile and have access to a list of generally accepted criteria on which to base one's diagnosis of the cause of hearing loss. In this way, the investigations carried out by different researchers would be easier to compare, and more reliable conclusions could be drawn from them.

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ABNORMALITIES OF LATERAL CARTILAGINOUS LAMINA AND LUMEN OF EUSTACHIAN TUBE IN CASES OF CLEFT PALATE

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Development of the lateral lamina (LL) of the eustachian tube (ET) cartilage was investigated in cleft palate (CP) cases and controls by measuring the areas of the LL and medial lamina (ML) of the ET cartilage in histologic cross section, and comparing the ratio of the area of LL to that of ML (LL/ML) in CP cases and controls. The degree to which the ET lumen appeared straight or C-shaped in cross section was also measured. Thirty temporal bones with the entire ET — 10 CP cases and 20 controls — were studied. The ratio of the areas of the ET cartilage was significantly smaller ($t = 2.903$, $p < .01$) and the curvature of the ET lumen was significantly less ($t = 2.080$, $p < .05$; $\chi^2 = 3.936$, $p < .05$) in CP cases than in controls. In addition, in all 30 CP cases and controls, the ratio of the areas of cartilage was significantly less in specimens with straight ET lumens than in those with C-shaped lumens ($t = 2.713$, $p < .02$). The results appear to show that the ET cartilages and lumens are anomalous in CP cases. These anomalies are considered to be important factors in active ventilatory dysfunction of the ET.

KEY WORDS — cleft palate, congenital malformation, eustachian tube lumen, lateral lamina of eustachian tube cartilage.

INTRODUCTION

Active ventilatory function (active opening) of the eustachian tube (ET) depends heavily upon functional cooperation between the tensor veli palatini muscle (TVPM) and the lateral lamina (LL) of the ET cartilage as well as the lateral membranous wall of the ET lumen. This observation is based on reports that the TVPM is the only muscle that actively opens the ET lumen[1-3] and on the results of our previous study[4] that show that the TVPM inserts into the most inferior portion (tip) of the LL of the ET cartilage in normal individuals. The ET lumen, therefore, is considered to open actively by lateral shifting of the lateral luminal wall together with contraction of the TVPM. These findings indicate that poor development of the LL of the ET cartilage, and/or poor insertion of the TVPM into the LL, could lead to impaired active opening of the ET. In addition, from our experience, the ET lumen is "C-shaped" in cross section in normal individuals,[5] with the concave side facing laterally. We believe this curvature is one of the important structural factors that affect the ET opening when the lateral wall of the ET is pulled laterally by the contraction of the TVPM.

TABLE 1. AGES AND ANOMALIES OTHER THAN CLEFT PALATE AND LIP IN STUDIED CLEFT PALATE CASES

Age	*Other Anomalies*
32 gw	Microtia, atresia auris, micrognathia, phocomelia
36 gw	Low-set ears, polydactyly
1 d	
1 d	Low-set and posteriorly rotated ears, ventricular septal defect
3 d	Trisomy 13
10 d	
3 wk	Trisomy 13
1 mo	Low-set ears, ectrodactyly
1 y	Holoprosencephaly
3 y	Hydrocephalus

gw — gestational weeks.

On the other hand, in individuals with cleft palate (CP) as well as abnormal tubal compliance,[1,6,7] anomalies in the "salpingopharyngeal" portion — hypoplastic levator veli palatini muscle (LVPM) and/or TVPM; abnormal course of the LVPM and its abnormal insertion into the palate — have been cited as one cause of ET dysfunction.[8-13] Such anomalies are known to predispose to middle ear disease such as otitis media with effusion.[14,15]

In our previous study, it was found that insertion

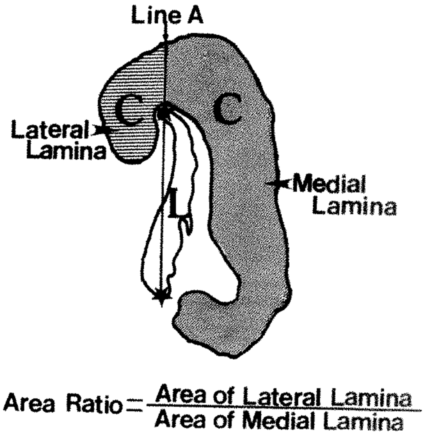

Fig 1. Schematic representation of method used to obtain ratio of lateral eustachian tube lamina to area of medial lamina. Eustachian tube cartilage (C) was divided into lateral lamina and medial lamina by line (line A) connecting most distant two points on margin of eustachian tube lumen (L).

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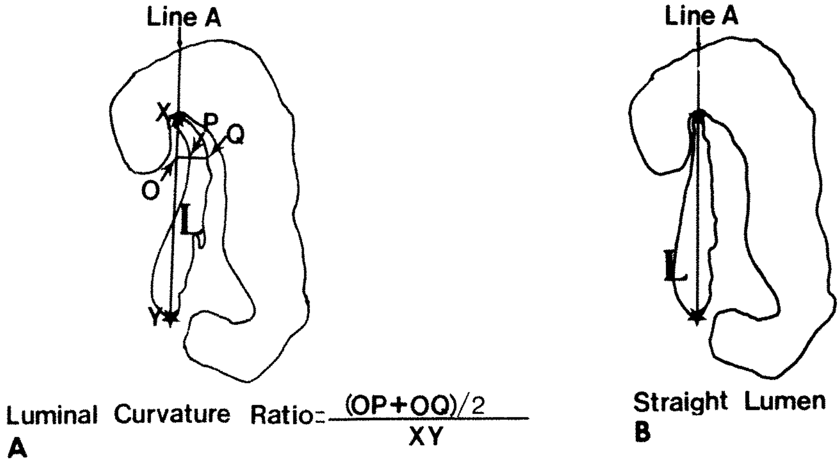

Fig 2. A) Schematic representation of method used to obtain luminal curvature ratio. Point P, on lateral margin of eustachian tube lumen (L) most distant from line A, was defined. From point P, line was extended perpendicular to line A to point O on line A and to point Q on medial margin of L. X, Y — most distant two points on margin of eustachian tube lumen. B) In cases in which entire lateral margin of ET lumen (L) was lateral to line A, L was classified as "straight" and no luminal curvature ratio could be obtained.

of the TVPM into the tip portion of the LL was reduced in CP cases compared to that in controls.[4] In this study, we sought to elucidate one of the causes of ET dysfunction in CP cases, by comparing development of the LL and the ET lumen in CP cases and in controls.

METHODS AND MATERIALS

Thirty human temporal bone specimens (Table 1) with the entire ET and its accessory structures were examined. Ten specimens were from children with CP (32 gestational weeks to 3 years old), and 20 specimens were from children without malformation (33 gestational weeks to 2 years old). All specimens were processed histologically according to the technique described by Sando et al.[16] Serial 30-μm-thick histology sections were cut vertically in the plane perpendicular to the long axis of the ET. Every 20th section was stained with hematoxylin and eosin, and a section from the midcartilaginous portion of each specimen was evaluated under light microscopy and the following measurements were obtained.

1. Cross-sectional areas of the LL and medial lamina (ML), measured with the digitizer (Hitachi HDG-1111), and the ratio of the areas as defined in Fig 1 (area ratio: LL/ML).
2. The curvature of the ET lumen in cross section as the ratio of the distance of the mean deviation of the ET lumen due to its curvature to the length of the long axis of the ET lumen (luminal curvature ratio) shown in Fig 2A (luminal curvature ratio) and 2B (straight lumen).
3. The area ratios of cases with straight ET lumens and those with C-shaped ET lumens.

RESULTS

A photomicrograph of a control case is shown in Fig 3A. The cross-sectional area of the LL is large. The ET lumen is concave laterally, so that a C-

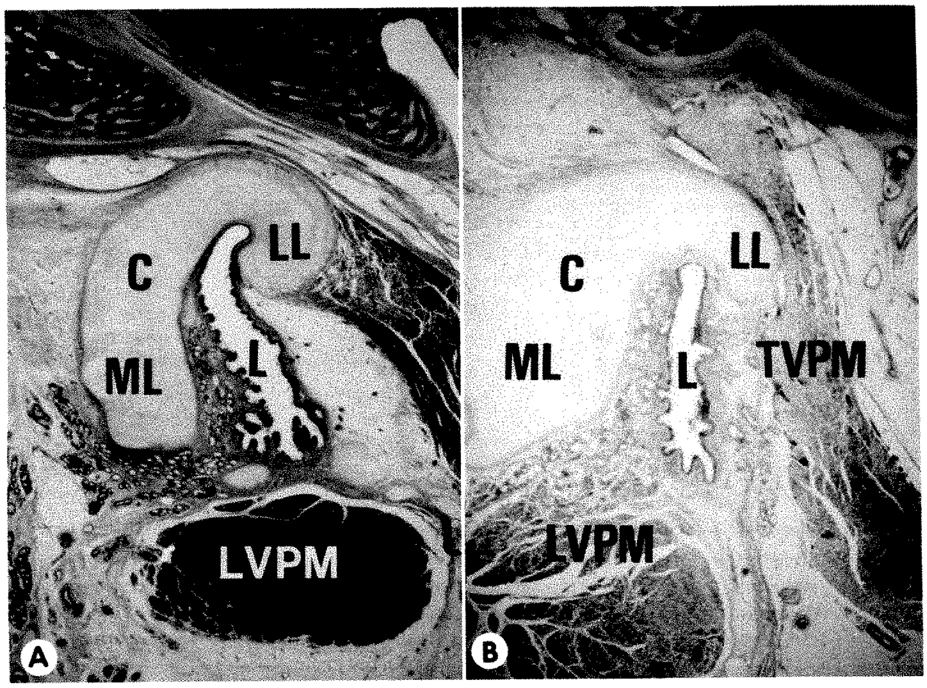

Fig 3. Photomicrographs of cross sections through midcartilaginous portion of eustachian tube (ET) of A) control (6-week-old girl) and B) cleft palate case (7-week-old boy) (H & E). C — ET cartilage, L — ET lumen, LL — lateral lamina of ET cartilage, LVPM — levator veli palatini muscle, ML — medial lamina of ET cartilage, TVPM — tensor veli palatini muscle.

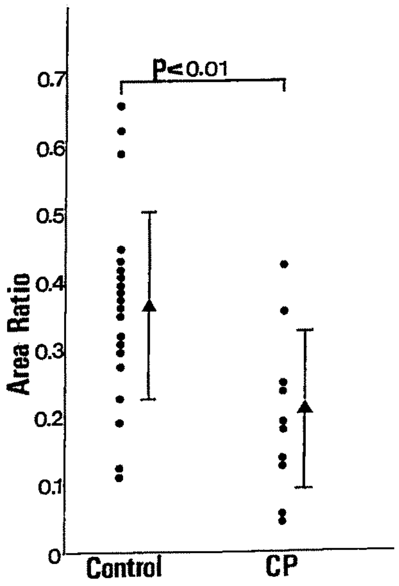

Fig 4. Area ratios of cleft palate (CP) cases and controls.

shaped cross-sectional profile results. In this particular case, the area ratio was 0.6561 and the luminal curvature ratio was 0.17. A photomicrograph of a CP case is shown in Fig 3B. The cross-sectional area of the LL was quite small and the ET lumen looked slitlike in cross section. In this particular case, the area ratio was 0.1833, and although the luminal curvature ratio could not be obtained, the lumen was classified as "straight."

The area ratio (Fig 4) was significantly smaller in CP cases than in controls ($t = 2.903$, $p < .01$). The straight ET lumens were significantly more often seen in CP cases than in controls (χ^2 test, $t = 3.936$, $p < .05$; Table 2). The luminal curvature ratio (Fig 5A) obtained was also significantly smaller in 5 CP cases than in 18 controls ($t = 2.080$, $p < .05$).

TABLE 2. SHAPE OF EUSTACHIAN TUBE LUMEN IN CLEFT PALATE CASES AND CONTROLS

	C-shaped Lumen	*Straight Lumen*	*Total*
Cleft palate cases	5	5	10
Controls	18	2	20
Total	23	7	30

$t = 3.936$, $p < .05$.

Overall, the area ratio was significantly smaller in 7 specimens with straight ET lumens than in the remaining 23 specimens with C-shaped ET lumens ($t = 2.713$, $p < .02$; Fig 5B).

DISCUSSION

In this study, a line ("line A" in Figs 1 and 2) was used to connect the most distant two points on the ET lumen in cross section to study the area ratio and the luminal curvature ratio of the ET. Because line A represents the main axis of the ET lumen in cross section, the ML and LL could be operationally defined as those parts lying medial to and lateral to the longitudinal axis of the tube. It was also advantageous that line A could be drawn consistently in each case.

Six of the 10 CP cases used in this study accompanied other anomalies in the cranium. These 6 CP cases, however, did not show a specific tendency either in the area ratio or the luminal curvature ratio. For instance, half (3 cases) of those 6 CP cases had a straight ET lumen, while the remaining half (3 cases) had a curved C-shaped ET lumen. The anomalies other than CP, therefore, do not seem to affect our present results.

The results of measuring the areas and calculating the area ratios of the LL to ML in CP cases and controls showed the CP cases to have less developed

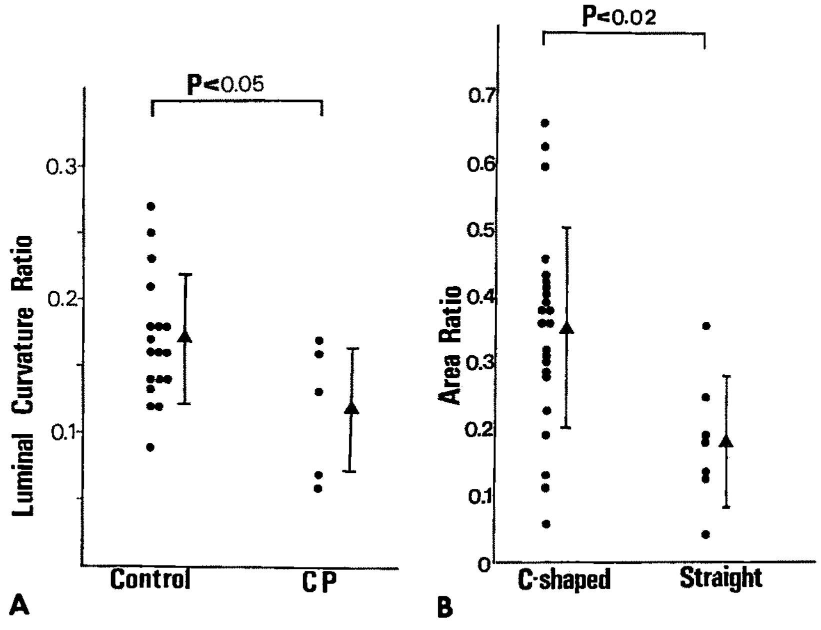

Fig 5. A) Luminal curvature ratios of cleft palate (CP) cases and controls. B) Area ratios of cases with C-shaped eustachian tube lumens and those with straight lumens.

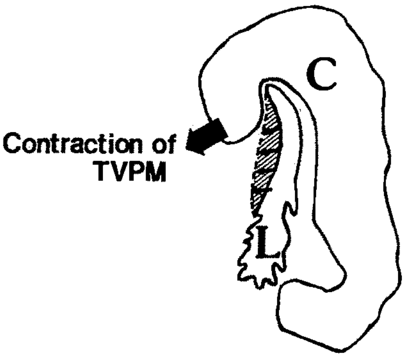

Fig 6. Schematic representation showing relationships between curvature of eustachian tube (ET) lumen (L) and lateral lamina of ET cartilage (C). Lateral wall of ET seems easily to move laterally (small arrows) in response to lateral movement (large arrow) of lateral lamina due to contraction of tensor veli palatini muscle (TVPM). This movement expands space (shaded area) in ET lumen.

ETs compared to the controls. This, together with insufficient insertion of the TVPM into the tip portion of the LL in CP cases[4] and in conjunction with other anomalies around the ET and nasopharynx,[8-13] seems to be the structural basis for insufficient active opening of the ET in CP cases.

A C-shaped ET lumen appears to be more functional than a straight lumen to accomplish active opening of the tube; this is especially true for the superior portion, in which movement of the concave lateral wall of the ET provides easy movement of the tube wall laterally with contraction of the TVPM (Fig 6). Less curvature of the ET lumen may be one of the factors hampering the efficient opening of the ET in CP cases. In addition to the opening failure, closing failure of the ET has been pointed out in CP cases,[15,17] in which the ET usually closes lightly and easily becomes patulous from time to time. Lack of sufficient development of the LL and luminal curvature may relate to a lack of a mass effect necessary for the tight closure of the tubal lumen in CP cases.

The ET develops gradually from the proximal portion of the first pharyngeal pouch, and eventually, during the 4th gestational month (GM), the ET lumen becomes slitlike in shape, according to Proctor.[18] According to Wolff[19] and Altmann,[20] "medial and lateral parts of the cartilaginous wall of the ET" (ML and LL) develop in the fourth and fifth GMs, respectively. Another, more recent study[21] also reported that ET cartilage, by the fifth GM, increases in size, growing laterally and superiorly, to abut and override the ET lumen. It is speculated that as the LL develops, beginning around the fifth GM as mentioned above, the LL may influence the degree to which the tube curves. Findings from these embryologic studies and our observations that seven cases in this study in which the ET lumen was straight had significantly smaller LL than controls both indicate that abnormalities in the LL and the ET lumen are the result of impaired development of these structures during fetal life and that these abnormalities are closely associated with each other and with active ventilatory dysfunction of the ET in CP cases.

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EVALUATION OF THE TEMPORAL BONES OF A MULTICHANNEL COCHLEAR IMPLANT PATIENT

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In this report we detail the temporal bone findings of a 57-year-old patient who underwent placement of a Nucleus 22-channel electrode 7 months prior to his death. Audiometric testing postoperatively demonstrated suprasegmental speech cue discrimination only. Histologic evaluation of the cochleas revealed significant sensorineural survival except in the region of the basal turn of the implanted cochlea adjacent to the implant. There was no significant pathologic condition attributable to the operation or the electrode in areas remote from the basal turn of the cochlea.

KEY WORDS — cochlear implant, multichannel electrode, temporal bone.

INTRODUCTION

In spite of the significance of knowing the effects of implantation and stimulation on human cochleas, there have been few reports on the tissue response after human cochlear implantation.[1-5] Clark et al[5] recently presented the first complete report of temporal bone findings from a patient with a Nucleus multichannel implant. This paper summarizes the findings in a patient who had received a unilateral implant of a Nucleus 22-channel scala tympani electrode. Seven months after implantation the patient died of metastatic colon cancer.

CLINICAL HISTORY

Preoperative. A 57-year-old laborer had experienced recurrent episodes of bilateral otitis throughout his childhood and teenage years. He had lost useful hearing on the right side some months following a modified radical mastoidectomy in 1947. In 1952 the patient experienced onset of progressive hearing loss in the left ear. He wore a hearing aid on the left side from 1955 until 1983, when he ceased to obtain benefit from the aid.

High-resolution computed tomography scanning of the temporal bones demonstrated a canal wall down mastoid cavity on the right side with a patent cochlea. The left temporal bone demonstrated an aerated, albeit contracted, mastoid with a patent cochlea.

Implantation. On July 17, 1987, a left cochlear implantation was performed through a transmastoid approach. Exposure of the round window niche and promontory was obtained through the facial recess approach. The electrode was inserted through a cochleotomy created anterior and inferior to the tegmen of the round window niche. The electrode array was rotated clockwise during passage to facilitate insertion. Twenty-six of 32 rings (19.5-mm insertion) were inserted into the cochlea. (Six stiffening rings remained outside of the cochlea.) The connecting lead was secured in its position with two Dacron ties anchored to the mastoid cortex posteriorly and inferiorly. Twenty-four hours following the operation, a transorbital anterior-posterior plain film demonstrated the implant to be in the expected surgical position (Fig 1A).

Postoperative. One month following surgical implantation, the patient returned for activation of the implant. Activation of the basilar electrodes failed to produce an auditory sensation. Repeat transorbital films demonstrated significant migration of the implanted array (Fig 1B). Approximately 13 electrodes (9.75 mm) appeared to be within the cochlea. The shift in the electrode array was associated with movement of that portion of the connecting lead between the mastoid tip and the cochlear insertion site.

The patient used the implant daily after activation and claimed subjective benefit from it. Subsequently, he developed jaundice and lethargy secondary to metastatic colon cancer. Seven months following the implantation procedure, the patient died of liver failure and respiratory insufficiency.

AUDIOMETRY

Preoperative. Pure tone earphone audiometry elic-

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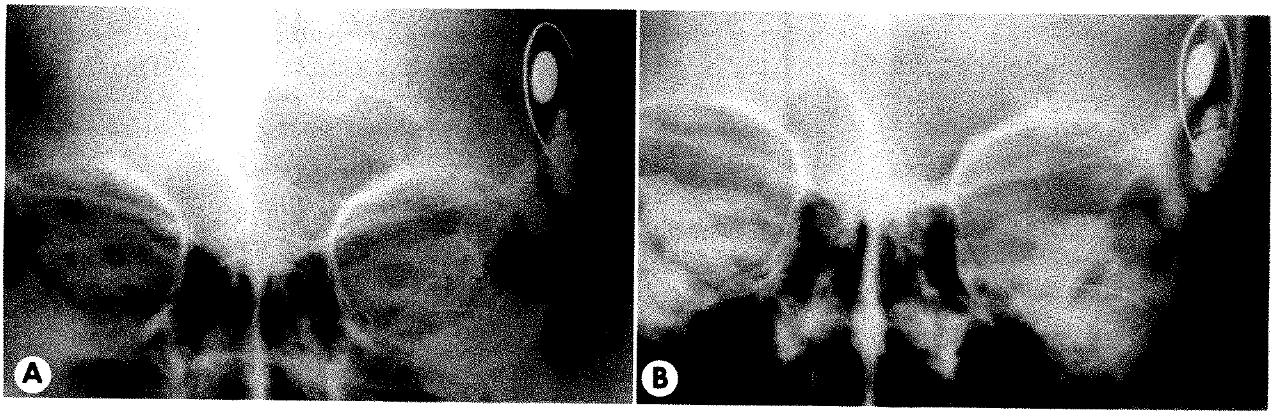

Fig 1. Transorbital anterior-posterior x-ray films taken after placement of implant. A) Twenty-four hours postoperatively, electrode can be seen coiled in basal turn of cochlea. B) One month postoperatively, electrode has shifted and extends only into first portion of basal turn.

ited no response at any frequency bilaterally. Figure 2 shows the audiograms of the patient in 1982 and 1986. In the left ear a speech discrimination threshold of 90 dB was obtained, and no response was elicited in the right ear.

Although hearing was slightly improved when a hearing aid was used in the left ear, no speech discrimination resulted. Aided speech-tracking scores (eight words per minute) were not substantially better than speech-tracking scores in the unaided condition (five words per minute).

The promontory of the tympanic cavity was stimulated with square wave pulses of 2.5, 5, 10, and 20 milliseconds per phase duration, in 0.5-second bursts at a rate of 1/s. Auditory sensations were obtained in both ears for all stimuli. Thresholds and maximum comfortable loudness levels were lower for the left ear than for the right. In addition, dynamic ranges were larger for the left ear.

Gap detection thresholds in response to biphasic pulse stimuli (20/s, 5 milliseconds per phase) were 100 and 150 milliseconds for the left and right ears, respectively. Temporal difference limens of 150 and 250 milliseconds were obtained in the left and right ears, respectively. Caloric stimulation of the vestibular labyrinth with 30°C water documented normal and symmetric responses bilaterally.

Postoperative. Subjectively the patient noted improved environmental contact and communication. He also related substantial reduction in his left ear tinnitus and maintained activation of the implant for an average of 14 hours per day, 7 days a week. This resulted in approximately 2,600 hours of stimulation. Auditory thresholds in the sound field with use of the prosthesis are shown in Table 1. Although the patient was unable to discriminate open-set speech, he reported that use of his implant substantially improved his ability to lip-read. This may have resulted from the suprasegmental speech information that the implant provided.

Selected tests from the Minimum Auditory Capabilities battery[6] (question/statement, noise/voice, accent, and four-choice spondee tests) administered postoperatively are compared with preoperative re-

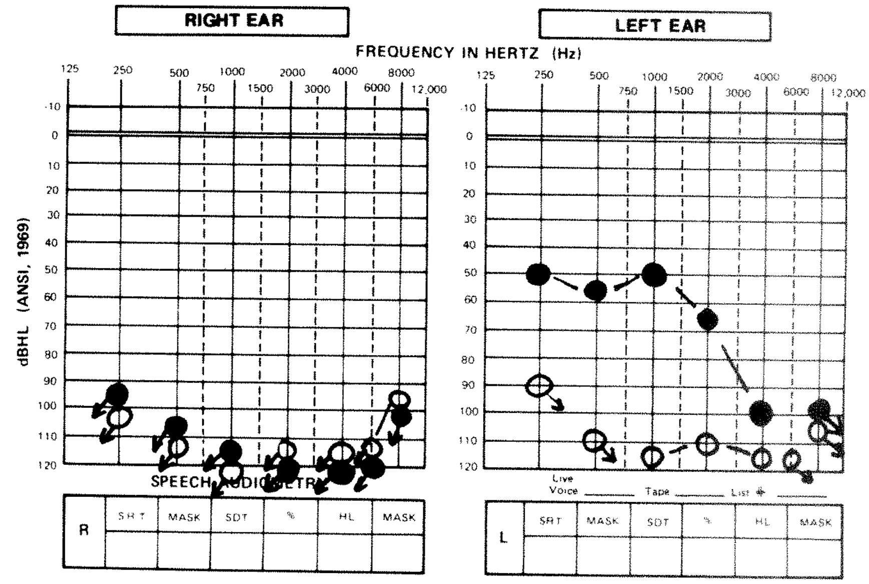

Fig 2. Superimposed pure tone audiograms from 1982 (closed circles) and 1986 (open circles) show difference in levels in middle and low frequencies of left ear. There was no conductive component to patient's hearing loss at any time.

TABLE 1. WARBLE-TONE THRESHOLDS IN DECIBELS HEARING LEVEL IN SOUND FIELD WITH HEARING AID AND COCHLEAR IMPLANT

	Signal Frequency (Hz)					
Condition	*250*	*500*	*1,000*	*2,000*	*3,000*	*4,000*
Aided right	>95	>105	110	>110	105	105
Aided left	>95	>105	100	95	>120	115
Implanted left		50	45	40	40	45

sults (Table 2). The question/statement test score revealed discrimination of intonation patterns. The patient's scores for the four-choice spondee, accent, and noise/voice tests were not above chance level and showed no substantial improvement compared to the preimplant test levels.

HISTOLOGIC METHODS

Twelve hours after the patient's death his temporal bones were harvested and perfused with glutaraldehyde. The implant was gently removed from the scala tympani to facilitate tissue fixation and sectioning. The temporal bones were postfixed with 1% osmium tetroxide in physiologic buffer. The temporal bone was drilled away until only the cochlea and vestibule remained in a thin shell of bone. Decalcification was completed over a 2-month period in 2% ethylenediaminetetraacetic acid. The cochlea was embedded in epoxy resin and sectioned (6 μm thick) in a near-sagittal plane parallel to the modiolus.

Hair cell survival was estimated from the 10 sections that were nearest the midmodiolar plane. For each section, it was assumed that there should be one inner hair cell and three outer hair cells present in each turn of the cochlea. The number of hair cells actually present in each turn was divided by this number, and an estimate of the percentage of surviving inner and outer hair cells was obtained.

By using the same 10 sections, spiral ganglion cell densities were calculated with a computer program capable of both cell counting and area estimation. Spiral ganglion cells were judged to be present if there was an identifiable nucleolus. Spiral ganglion cell density was determined by dividing the cell count by the tissue volume (product of the area estimation and the section thickness) and multiplying by the correction factor introduced by Konigsmark[7] and used by Nadol.[8] In our preparation, the correction factor was 6 μm/8 μm (0.75), with 6 μm representing the section thickness and 8 μm representing the nucleolus diameter (2 μm) plus the section thickness (6 μm).

RESULTS

Gross. In the implanted left ear, filmy adhesions surrounded the implanted receiver-stimulator and connecting lead within the mastoid defect. At the time of removal, 13 active electrodes on the implant were found internal to the round window membrane.

TABLE 2. PERFORMANCE ON FOUR SPEECH TESTS WITH HEARING AID AND COCHLEAR IMPLANT IN LEFT EAR

Test	*Hearing Aid*	*Activated Implant*
Question/statement	40	70*
Noise/voice	60	63
Accent	10	30
Four-choice spondee	0	25

*Significant at .05 confidence level.

Histologic: Right (Unimplanted) Ear. Large numbers of inner and outer hair cells were observed. Surviving hair cell populations decreased from base to apex with substantial decrements in outer hair cell populations (Table 3).

In general, the outer hair cells appeared well preserved and were easy to distinguish (Fig 3). Some vacuolization and postmortem changes of the inner hair cells were found. Supporting cells were present throughout the entire length of the basilar membrane.

The spiral ligament, tectorial membrane, and Reissner's membrane appeared normal. The cells composing the stria vascularis were poorly defined, but the stria was of normal thickness and the vasculature was not significantly altered.

The spiral ganglion cell densities for the right ear are shown in Table 3. Survival varied from 30% to 54% of normal,[8] with higher densities present in the base. The spiral ganglion cells showed postmortem vacuolization but were easily identified (Fig 4). Although the peripheral dendritic processes in the osseous spiral lamina were not quantified, they appeared to be present in proportions similar to the adjacent spiral ganglion cells.

Histologic: Left (Implanted) Ear. A dense connective tissue cuff (Fig 5) was evident along the implant track throughout the length of the implanted basal turn (9 mm) and extended 3 mm outside the round window. Histologically, this track was lined with a dense acellular fibrous sheath approximately 20 μm in width. This fibrotic cuff was surrounded by loose, less-dense areolar tissue that occupied less

TABLE 3. SURVIVING HAIR CELL POPULATIONS

Distance From Round Window (mm)	*Spiral Ganglion Cell Density (cells/0.001 mm³ ±SD)*	*Percent of Control**	*Inner Hair Cells (%)*†	*Outer Hair Cells (%)*†
Unimplanted right ear				
6	24.4 ±6.3	54	50	90
15	14.3 ±3.7	30	50	35
22	16.9 ±4.2	34	40	30
Apical (26-32)	16.0 ±6.5	46	20	5
Implanted left ear				
6	4.45±1.3	9.9	0	0
15	19.0 ±5.4	40	30	60
22	23.2 ±6.3	50	40	15
Apical (26-32)	34.0 ±5.6	97	20	5

*Compared to controls from Nadol.[8]

†Estimated.

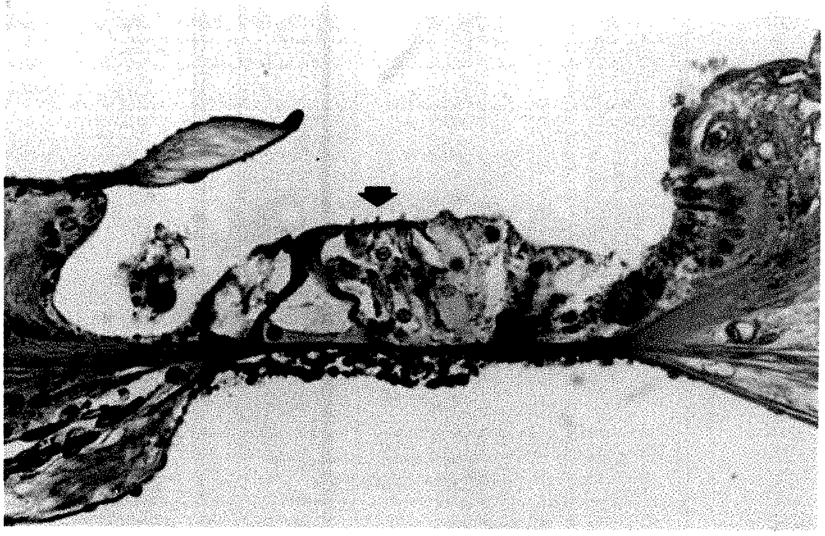

Fig 3. Photomicrograph of organ of Corti in basal turn of right (unimplanted) ear shows stereocilia (arrow) of three outer hair cells (toluidine blue, original x300). Inner hair cell is not well visualized.

of the scala tympani as it progressed away from the round window. In the proximal basal implanted turn, this tissue appeared to fill the scala tympani. Aggregates of inflammatory cells within the loose areolar tissue were present in areas of the scala tympani remote from the electrode sheath and the endosteal surface. Foci of ossified tissue were apparent within some of these inflammatory cell congregates (Fig 5).

Reissner's membrane was disrupted in the proximal 3 mm of the implanted basal turn, with a short segment of the membrane attached to the lateral wall. We attributed its discontinuity to preparation artifact, perhaps as a result of scalar perfusion.

Spiral ligament distortion was apparent within the first 3 mm of the round window and at a distance of 9 to 10 mm from the round window. The defect within 3 mm was located in the central portion of the spiral ligament and did not communicate with the scala tympani (Fig 6A). The defect at 9 to 10 mm can be characterized as a compression of the spiral ligament (Fig 6B). This was the area of the final resting position (after the first postoperative month) of the tip of the electrode. The basilar membrane was intact in both areas in which the spiral ligament was distorted. In the basal turn, the stria vascularis was thinner, with a smoother scalar border than in the more apical turns. It was uncertain if this was related to the implant or if it was simply a fixation artifact.

A basilar membrane defect was apparent 4 to 5 mm from the round window. The defect prolapsed into the scala tympani and was lined with a thin epithelial layer (Fig 7). The organ of Corti (including tectorial membrane, spiral limbus, and supporting cells) was absent in this region of the basilar membrane defect. The osseous spiral lamina was intact throughout.

Table 3 also summarizes the hair cell findings in the left ear. The sensory and supporting cells were absent throughout the basal 11 mm of the implanted cochlea. It is at the junction of the basal and middle thirds of the basal turn that the cellular elements of the basilar membrane appear (Fig 8). Apically, the hair cell findings were similar to those in the unimplanted ear.

Except for the basal turn adjacent to the implanted portion of the scala tympani, spiral ganglion survival was greater in the implanted ear (Table 3). Adjacent to the implant there was extreme loss of spiral

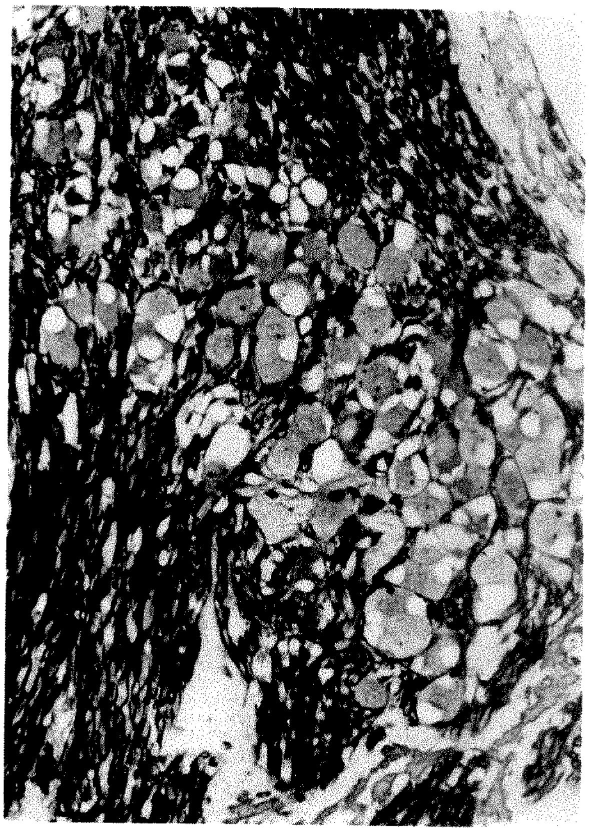

Fig 4. Photomicrograph shows spiral ganglion cells in apex of right (unimplanted) ear (toluidine blue, original x200). Postmortem intracellular vacuolization can be seen.

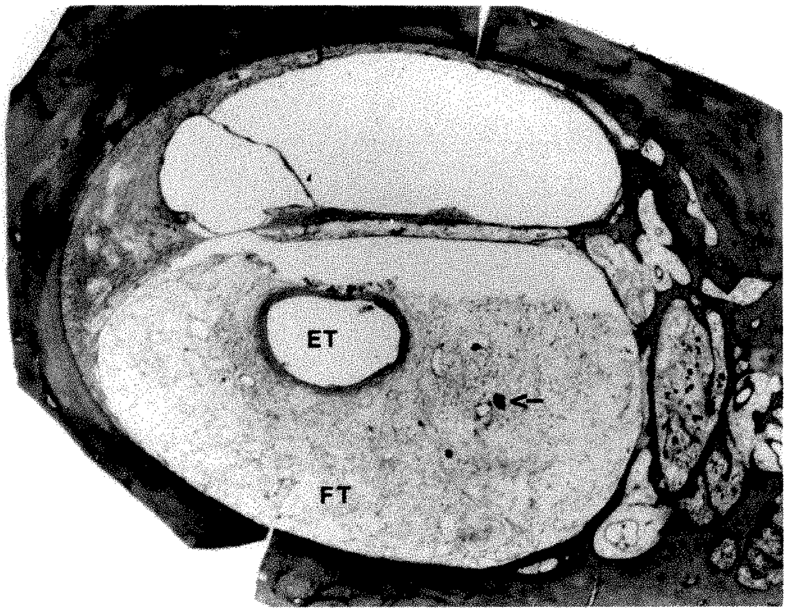

Fig 5. Fibrous tissue (FT) filling most of scala tympani in basal turn along electrode track (ET) (toluidine blue, original x200). Thick, dense acellular tissue layer (20 μm) immediately surrounds track. Away from track there is focus of ossification surrounding congregation of inflammatory cells (arrow).

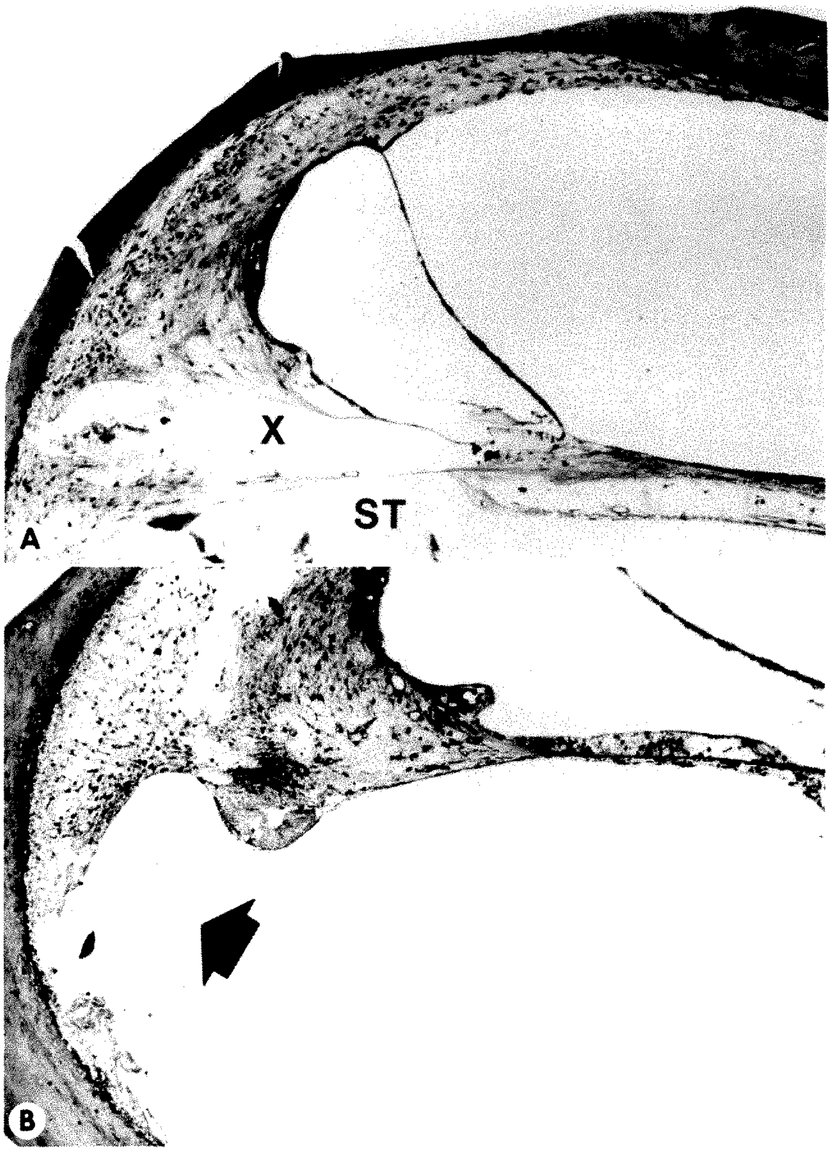

Fig 6. Spiral ligament distortion in basal turn of left (implanted) ear (toluidine blue, original x80). A) More proximal defect (X) appeared at approximately 3 mm from round window. Defect is outside of scala tympani (ST) where electrode was present. B) Second disruption was compression defect (arrow) 9 to 10 mm from round window. This is area where tip of electrode was present.

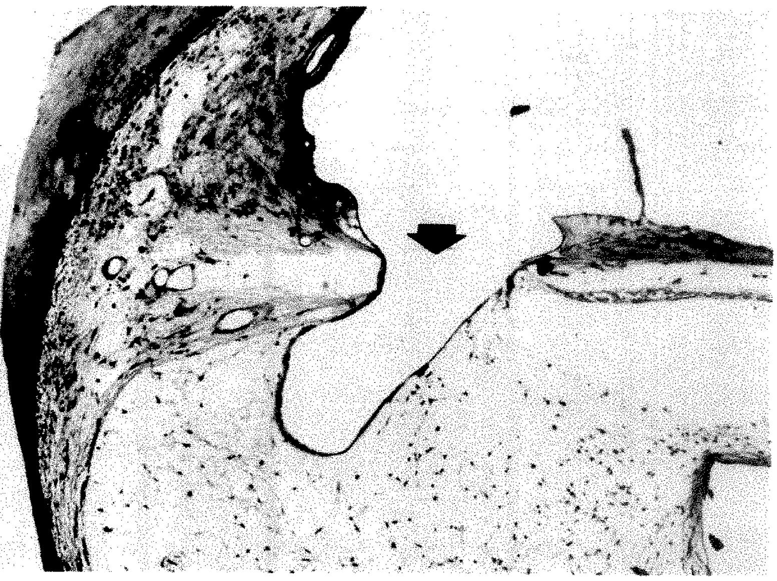

Fig 7. Defect in basilar membrane (arrow) in basal turn of left ear, 4 to 5 mm from round window (toluidine blue, original x80).

ganglion cell populations (9.9% of controls[8]). In this ear, as in the right one, the peripheral dendritic processes appeared to be present in proportion to the adjacent spiral ganglion cells.

DISCUSSION

The implantation procedure was uncomplicated, and all 22 of the active electrodes, in addition to 6 of the 10 stiffening rings, were inserted into the cochlea. One month later, a significant shift of the electrode out of the cochlea was apparent. The reason for and exact time of this event are unknown. It does appear, however, that there was a rotation of the electrode between the distal point of fixation (the inferior Dacron tie) and the round window. This may have resulted from relaxation of a twist of the connecting lead that may have resulted from the clockwise rotation with insertion. This occurrence is unique in our experience.

Areolar connective tissue occupied the scala tympani and there was an absence of inflammatory reaction immediately adjacent to the electrode. There were several areas of connective cell aggregates remote from the electrode sheath. This may represent the reaction to bone fragments or dust introduced at the time of operation.

In the implanted left cochlea, the basic structure of the organ of Corti was maintained in the apical portion of the basal turn, while it was absent in the basal portion of the basal turn. At a distance of approximately 11 mm from the round window the elements of the organ of Corti appeared on the basilar membrane. Changes in the cochlea possibly attributable to implantation were only observed in the area where the implant was present chronically (with stimulation). The portion of the basal turn where the electrode was placed initially, but displaced soon after surgery, was not significantly altered in comparison to the remainder of the cochlea and the contralateral cochlea.

A considerable surviving hair cell population was seen in both implanted and unimplanted cochleas. While remaining hair cells are often seen in cochleas of deaf patients,[9] the populations identified in the present patient were considerably larger. One would expect some functional hearing based on the number and position of the remaining sensory and neural elements. The hearing impairment may have had a central auditory system component.

The presence of hair cells in any significant number has not been observed in previous histologic studies of implanted ears. Clark et al[5] found no remaining hair cells in either the implanted or contralateral ear. Johnsson et al,[4] in their evaluation of a patient implanted bilaterally, found no hair cells in one ear and 16 in the opposite ear.

The loss of the organ of Corti may also have been the result of basilar membrane injury. The basilar membrane defect observed at 4 mm from the round window, if patent, may have resulted in extensive sensorineural damage. Dimitrov et al[10] demonstrated extensive sensorineural damage resulting from basilar membrane fistulas in rabbits.

We assume the well-healed defect of the basilar membrane (Fig 7) occurred either at the time of implantation or at the time of shifting of the implant in the early postoperative period. The defect in the basilar membrane could represent a traction injury if the electrode adhered to the basilar membrane prior to its migration in the first month postoperatively. This portion of the basilar membrane may have prolapsed into the scala tympani. In addition, basilar membrane injury may have resulted from buckling of the electrode at the time of insertion.[11]

The spiral ligament injuries in the implanted ear are similar to those described by other authors. Shepherd et al[12] placed implants in fresh cadaver

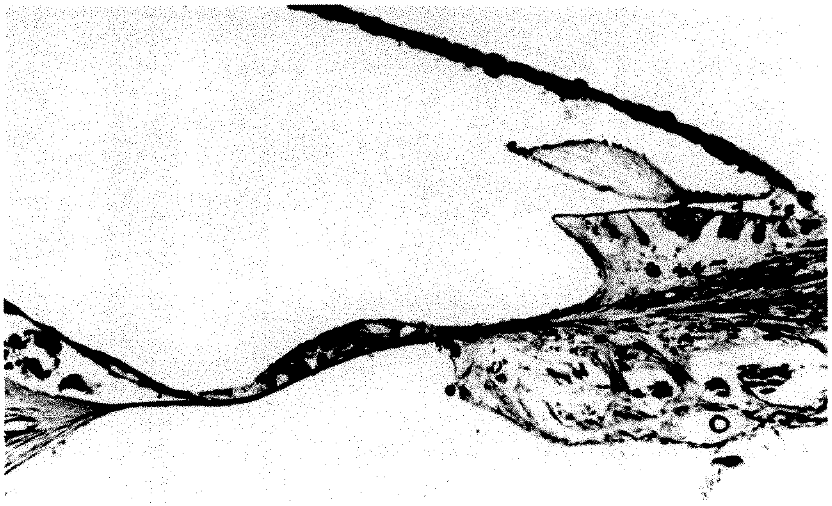

Fig 8. First cellular elements appear on basilar membrane at distance of 11 mm from round window (toluidine blue, original x200).

temporal bones and found that the most common injury was to the spiral ligament 7 to 11 mm from the round window. Kennedy,[11] in a similar experiment, found that damage to the spiral ligament occurred at the point of first resistance, which was 10 to 17 mm from the round window. Schindler and Bjorkroth[13] demonstrated that regions just apical to the hook are most prone to damage at the time of insertion, although they demonstrated that fractures of the osseous spiral lamina were characteristic.

The loss of the organ of Corti in the basal turn was associated with a marked decrease in spiral ganglion cell population in the corresponding canal of Rosenthal. It is impossible to determine if the cause of the spiral ganglion cell loss was secondary to the loss of the sensory epithelia or if it was a direct result of the underlying pathologic condition. The loss of sensory cells, peripheral neuronal processes, and spiral ganglion cells in the implanted left basal turn may have been related to the presence of the electrode, the implantation procedure, subsequent shifting of the electrode array, and/or stimulation mediated by the implant. Alternatively, audiometric results prior to the onset of profound deafness (Fig 2) suggest that regions of the left cochlea subserving high frequencies were nonfunctional 5 years prior to implantation. Therefore, we cannot exclude the possibility that the underlying pathologic condition that led to deafness may have contributed to the regional sensory and neural changes seen.

Otte et al[14] concluded that at least 10,000 spiral ganglion cells, with 3,000 being in the apical 10 mm (the most critical area for providing speech information), were required for speech discrimination. Although the present patient was clinically deaf, spiral ganglion cell survival ranged from 30% to 54% in the unimplanted ear. Given an average spiral ganglion cell population of >28,000 in normal adults,[14] the population of spiral ganglion cells in this patient can be estimated to be at least 10,000, with more than 3,000 in the apex. Structurally, it is difficult to explain the profound deafness in this patient preoperatively. This may reflect processing abnormalities that were independent of the surviving peripheral sensorineural elements and/or inadequate stimulation of these elements. In addition, analysis of the auditory brain stem is currently under way to determine if central pathologic changes contributed to the clinical picture.

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Ann Otol Rhinol Laryngol 100:1991

AIRWAY OBSTRUCTION AND HIGH-FREQUENCY JET VENTILATION DURING LARYNGOSCOPY

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High-frequency jet ventilation has been reported as an effective method of ventilation during laryngoscopy, but may expose the patient to the risks of barotrauma or alveolar hypoventilation. The aim of the study was to evaluate the determining factors of pulmonary complications under high-frequency jet ventilation in 83 patients undergoing laryngoscopy for upper airway cancer. Pulmonary distention was mainly influenced by upper airway obstruction score ($p = .0001$), while patients with chronic obstructive pulmonary disease (COPD) did not suffer from gas trapping. Impaired gas exchange was predicted by increased weight ($p = .0001$), smaller injector diameter ($p = .02$), and lower airway obstruction ($p = .001$). Hypercapnia occurred in both upper and lower airway obstruction, while hypoxemia was principally observed in COPD patients. Emphasis is placed on monitoring by pulse oximetry, end-expiratory pressure, and PCO2 measurement, especially in patients with obesity, COPD, or upper airway obstruction.

KEY WORDS — airway obstruction, barotrauma, high-frequency jet ventilation, hypoventilation, laryngoscopy, lung volume measurements.

INTRODUCTION

High-frequency jet ventilation (HFJV) has been reported as an effective method of ventilation during laryngoscopy,[1] bronchoscopy, and surgery of the upper airway, including laser surgery. In these indications, HFJV offers the advantages of limitation of movements in the operative field as well as reduction of the competition for space by the use of small-diameter injectors. These injectors, initially described for manual Venturi jet ventilation, may be the lateral channel of the bronchoscope or the laryngoscope, or injection catheters inserted by different ways: orotracheal, nasotracheal, or percutaneous transtracheal.[2-6]

However, barotrauma has been described, especially during manual Venturi jet ventilation.[5,7-9] The occurrence of barotrauma may increase dramatically in the case of airway obstruction.[10,11] Airway obstruction is frequent in patients scheduled for laryngoscopy and may concern the upper airway or the lower airway in cases of chronic obstructive pulmonary disease (COPD). The risk of hypoventilation in such patients has been stressed by several authors.[1,12,13] The aim of this study was to quantify pulmonary distention and hypoventilation during HFJV for laryngoscopy and to evaluate the circumstances in which the respiratory parameters could be altered.

MATERIAL AND METHODS

Eighty-three patients (76 men and 7 women) undergoing laryngoscopy for upper airway cancer were included in this study after approval by our local ethics committee and informed consent from each patient.

During the preoperative consultation, summary data were collected: age, weight, and height. Computerized pulmonary function tests (Microspiro Hi 298, ETNA Laboratories, France) were performed in all the patients and gave the following data: vital capacity, forced expiratory volume in 1 second (FEV1), and peak expiratory flow. Patients were classified into two groups according to their previous respiratory history: in the COPD group, patients presented with asthma or productive cough over 3 consecutive months for at least 2 consecutive years; in the group without COPD (NCOPD), patients did not present these respiratory symptoms.

Anesthesia. Patients were premedicated with atropine sulfate (0.007 mg/kg) and diazepam (0.15 mg/kg), administered intramuscularly. Anesthesia was induced with an intravenous bolus of propofol (2 mg/kg) or methohexital sodium (3 mg/kg) with fentanyl citrate (0.10 to 0.15 mg) and maintained with a propofol or methohexital infusion. Muscle relaxation was achieved with vecuronium bromide (bolus of 0.08 mg/kg), readministered on demand under twitch control monitored with a peripheral nerve stimulator.

Ventilation. After induction of anesthesia, an injection catheter was introduced into the trachea: either a percutaneous narrow catheter (14 gauge, 10-cm length; $n = 64$) inserted through the cricothyroid membrane, or a nasotracheal catheter (12 gauge, 30-cm length; $n = 19$) introduced under laryngoscopy. In all cases, a tracheal injection of 2 mL of lidocaine hydrochloride 2% was given. The distal tip of all injectors was located 3 or 4 cm above the carina, as confirmed visually by the surgeon during the procedure. Patients were not intubated

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TABLE 1. SCORING OF UPPER AIRWAY OBSTRUCTION

Category	*Scoring Value*
Cancer localization	
Tongue	1
Oropharynx	2
Base of tongue	3
Hypopharynx	4
Larynx	5
Infiltration	
No	1
Yes	2
Exophytic character	
No	1
Mild	2
Major	3
Edema	
No	1
Yes	2
Fibrosis	
No	1
Yes	2

Final score was obtained by multiplying together values of each category.

and passive expiration was made possible by manually lifting the jaw. Then HFJV was started with the following settings: frequency = 2 Hz, inspiratory-expiratory ratio = 0.54, driving pressure = 3.7 bars, fractional percentage of oxygen in inspired gas (FiO_2) = 1. These settings were chosen on the basis of results of blood gas analysis in a pilot study (n = 12).

Measurements. Pulmonary distention was assessed by the change in pulmonary volume above apneic functional residual capacity (dFRC) and the end-expiratory pressure (EEP), which was found to be highly correlated with dFRC in a previous study.[14] For each patient dFRC was measured by external spirometry according to the method described by Rouby et al.[10] For this purpose, a 50-cm mercury strain gauge was strapped to the patient at the nipple level to measure changes in rib cage displacement. Frequency response and linearity were found to be satisfactory up to 15 Hz. In order to calibrate this gauge, the lungs were insufflated five to six times through the injector for 175 milliseconds (insufflation time corresponding to 2 Hz frequency and 0.54 inspiratory-expiratory ratio) at 4-second intervals, with the upper airway firmly occluded. In these conditions the entrained volume was nonexistent, and the displacement of the thorax was due only to the injected volume, which was measured for each catheter with a dry spirometer at the end of the study. Simultaneous airway pressure recording allowed the measurement of the static compliance. End-expiratory pressure was measured through the injector by using a second solenoid valve that connected the injection line to a piezoelectric transducer at the end of each expiration (ventilator GR 300, LSA, France).[14] End-expiratory pressure and dFRC were measured after 30 seconds of stable ventilation. Blood gases were sampled from the radial artery after 10 minutes of ventilation.

After these measurements, the surgeon performed the endoscopic examination. In order to quantify the degree of upper airway obstruction (UAO), a score was attributed to each of the following data: tumor localization, exophytic character, fibrosis, edema, and infiltration (Table 1). For each patient a UAO score was obtained by multiplying together the values of each characteristic.

Statistical Analysis. The relationships between preoperative values and intraoperative measurements (dFRC, EEP, blood gases) were first examined by univariate tests: least-square regression or Student's *t* test as appropriate. In the statistical analysis, UAO score was considered as a continuous variable.

Then four stepwise variable selections (Statgraphics, Rockville, Md) were used to examine the association between EEP, dFRC, PaO_2 or $PaCO_2$, and the preoperative characteristics that were found to be associated with these dependent vari-

TABLE 2. RESULTS OF STEPWISE VARIABLE ANALYSIS

	Preponderant Effect on Dependent Variable			
Dependent Variable	*Independent Variable*	*Coefficient*†	*p*	*None or Minor Effect*
dFRC	UAO score*	+5.1	.0001	Age, weight, height, FEV1, peak,* compliance, injector, clinical COPD*
PaO2	Weight*	−3.6	.0001	Age, height,* FEV1, peak, compliance, UAO score
	COPD*	0 (no COPD) −89 (COPD)	.0012	
	Injector*	0 (14 gauge) +57 (12 gauge)	.021	
PaCO2	FEV1	−0.19	.0001	Age, height,* peak,* compliance, UAO score, clinical COPD*
	Weight*	+0.26	.0024	
	Injector*	0 (14 gauge) −7.2 (12 gauge)	.0033	

dFRC — apneic functional residual capacity, UAO — upper airway obstruction, COPD — chronic obstructive pulmonary disease, FEV1 — forced expiratory volume in 1 second.

*Significant at $p < .10$ level of univariate test (least-square regression or Student's *t* test), which was condition for inclusion of variable in stepwise variable selection model.

†Plus or minus sign before each coefficient indicates direction in which independent variable affects dependent variable.

TABLE 3. PREOPERATIVE CHARACTERISTICS AND RESULTS ACCORDING TO UAO SCORE

	UAO Score <20 (n = 55)	*UAO Score ≥20 (n = 28)*
Age (y)	58 ± 9	61 ± 9
Weight (kg)	68 ± 13	70 ± 11
Height (cm)	169 ± 9	169 ± 7
FEV1 (% of predicted values)	77 ± 24	71 ± 17
Peak flow (% of predicted values)	66 ± 26	52 ± 18*
Compliance (mL/cm H2O)	72 ± 19	74 ± 21
COPD (No. of patients)	14	2
dFRC (mL)	151 ± 104	294 ± 214*
EEP (cm H2O)	1.22 ± 1.27	3.05 ± 2.51*
PaO2 (mm Hg)	389 ± 116	410 ± 99
PaCO2 (mm Hg)	44 ± 11	45 ± 10
Injector (No. of patients)		
Percutaneous	45	19
Nasotracheal	10	9

Values are mean ± SD.

EEP — end-expiratory pressure. Other abbreviations as in Table 2.

*Significant at $p<.05$ level (Student's *t* test or χ^2 test).

ables at the $p<.10$ level on univariate tests. The results of the stepwise variable selection were considered significant for $F>4$ at the $p<.05$ level.

RESULTS

As expected, EEP and dFRC were strongly correlated ($r = .90$) and dFRC was only considered in the stepwise regression analysis. The results of this multivariate analysis are shown in Table 2. The UAO score was the only predictive factor of pulmonary distention assessed by dFRC ($p = .0001$). The same result was obtained between the UAO score and EEP ($p = .0001$). To illustrate the results of multivariate analysis the patients were divided into two groups according to their UAO score. When dFRC exceeded 500 mL, the UAO score was always greater than 20, a level that can be arbitrarily chosen to identify UAO. Preoperative characteristics and results of dFRC, EEP, and blood gases according to a UAO score lower or higher than 20 are represented in Table 3. The mean dFRC and EEP were significantly increased when the score exceeded 20, while no difference was seen in blood gases. Figure 1 shows the recording of airway pressure and dFRC in a patient with UAO before and after release of the airway obstruction.

Low Pao2 was predicted by heavy weight, clinical COPD, and a narrow injector (Table 2). As shown in Fig 2, all the patients with a Pao2 lower than 250 mm Hg were in the COPD group.

A high Paco2 was predicted by a low FEV1, heavy weight, and a 14-gauge narrow injector (Table 2). Figure 3 shows the relationship between Paco2 and FEV1. As in Fig 2, patients were individualized according to the level of airway obstruction. The highest levels of Paco2 were observed in patients with COPD or a UAO score greater than 20.

Table 4 shows the preoperative characteristics and the results of dFRC, EEP, and blood gases in NCOPD and COPD patients. In COPD patients, Pao2 was lower and Paco2 higher than in NCOPD patients, even though dFRC and EEP were not statistically different.

Gas exchange was better in patients ventilated with a 12-gauge catheter than in those ventilated with a 14-gauge catheter: Paco2 = 37 ± 8 mm Hg versus 47 ± 11 mm Hg ($p = .001$) and Pao2 = 449 ± 77 mm Hg versus 378 ± 115 mm Hg, respectively ($p = .01$).

DISCUSSION

The operative assessment of the degree and level of airway obstruction is difficult, and conventional pulmonary function tests often fail to distinguish between upper and lower airway obstruction.[15,16]

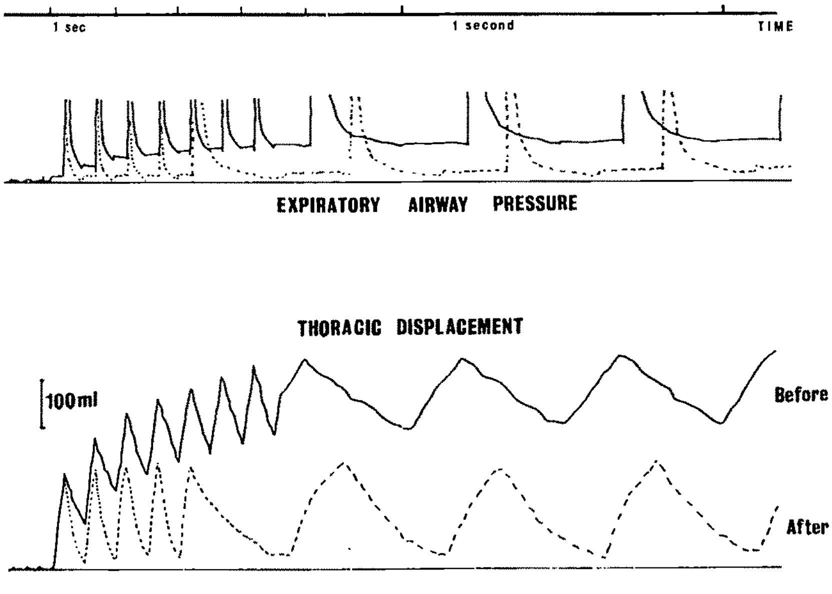

Fig 1. Recording of airway pressure and thoracic displacement in patient with upper airway obstruction. When ventilator was switched on, pulmonary gas trapping occurred (solid line). After release of airway obstruction by tracheal intubation, gas trapping became minimal (dotted line).

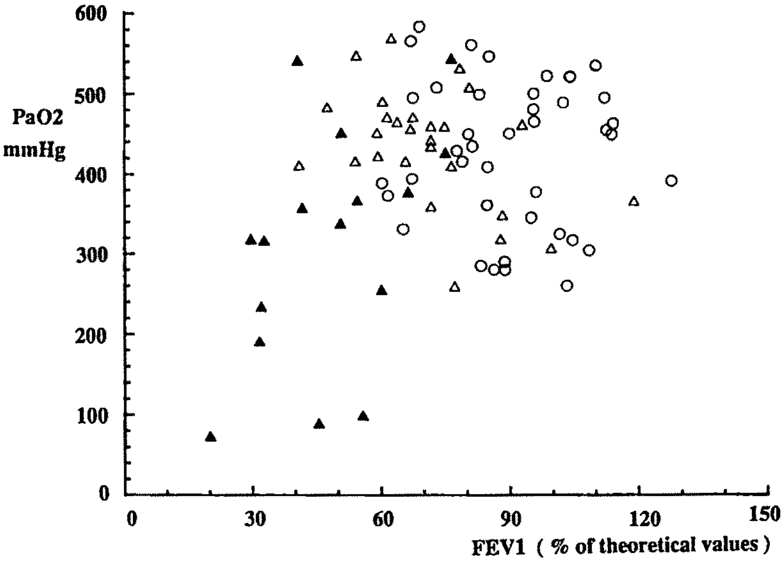

Fig 2. Relationship between PaO2 under high-frequency jet ventilation and preoperative forced expiratory volume in 1 second (FEV1). Circle — upper airway obstruction score < 20 and no chronic obstructive pulmonary disease, open triangle — upper airway obstruction score ≥ 20, filled triangle — chronic obstructive pulmonary disease. All patients with PaO2 lower than 250 mm Hg were in chronic obstructive pulmonary disease group.

For this reason the classification of patients as with or without COPD and the assessment of UAO were mainly clinical. The choice of an obstruction score is an anatomic approach to UAO, taking into account the localization, extent, and obstructive characteristics of a tumor. For example, a score value of 20 corresponds to a laryngeal localization with at least two obstructive characteristics.

The UAO score was the only factor predictive of increase in FRC in this study. The fact that UAO predisposes to barotrauma is consistent with the general opinion of clinicians.[8,12] Jet gas was injected downstream to the level of obstruction and all of it entered the lungs, while its exit was impeded by the reduction of the upper airway caliber, which thus induced intrapulmonary gas trapping. In practice, to avoid barotrauma, the continuous monitoring of EEP or external spirometry is indicated, especially in patients with UAO. Recently a jet ventilator has been built with a safety feature that stops injection when EEP exceeds a preset value,[14] and the use of this ventilator seems particularly interesting in UAO patients. More surprising is the fact that COPD does not appear to be a factor predisposing to an undesirable increase in FRC, contrary to widespread opinion.[10-12] The mean dFRC in COPD patients was relatively small in spite of a lower FEV1 than in other patients (Table 4). This is all the more surprising because a positive EEP effect has been described during conventional positive pressure ventilation with low frequency in patients with COPD.[17] This discrepancy may be explained by the fact that HFJV uses an open circuit. Since the increased inspiratory resistance was distal to the injection, gas compression occurred in the airway, the entry of gas into the alveoli was impeded, and part of the insufflated gas flowed back directly into the room air. This last phenomenon has been described previously in a lung model with elevated airway resistance[18] and during HFJV in anesthetized dogs.[19]

The weight of the patient was a risk factor for in-

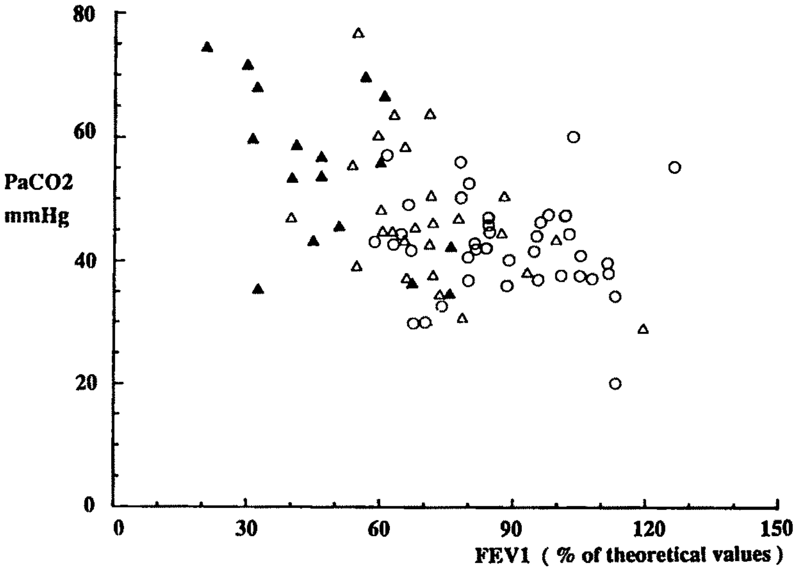

Fig 3. Relationship between PaCO2 under high-frequency jet ventilation and preoperative forced expiratory volume in 1 second (FEV1). Symbols as in Fig 2.

TABLE 4. PREOPERATIVE CHARACTERISTICS AND RESULTS IN PATIENTS WITH AND WITHOUT COPD

	No Clinical COPD (n = 67)	*Clinical COPD (n = 16)*
Age (y)	58±9	62±7
Weight (kg)	68±11	72±17
Height (cm)	169±9	171±8
FEV_1 (% of predicted values)	82±18	47±19*
Peak flow (% of predicted values)	66±23	39±16*
Compliance (mL/cm H_2O)	72±19	74±23
UAO score	18±14	12±12
dFRC (mL)	213±171	141±111
EEP (cm H_2O)	2.03±2.05	1.03±1.42
PaO_2 (mm Hg)	417±83	313±162*
$PaCO_2$ (mm Hg)	43±9	51±14*
Injector (No. of patients)		
Percutaneous	50	14
Nasotracheal	17	2

Values are mean±SD. Abbreviations as in Tables 2 and 3.

*Significant at $p < .05$ level (Student's *t* test or χ^2 test).

adequate ventilation, in terms of both hypercapnia and hypoxemia. Such a pattern has been described during conventional ventilation in obese patients and has been related to the inhomogeneity of the ventilation-perfusion distribution.[20]

Chronic obstructive pulmonary disease was associated with a lower PaO_2 than in NCOPD patients, and three COPD patients presented with severe hypoxemia ($PaO_2 < 100$ mm Hg), although FiO_2 was equal to 1. Similar results have been reported previously under HFJV.[1] This relative ineffectiveness of HFJV in COPD patients may be related to a ventilation-perfusion mismatch. Use of HFJV in patients with COPD should be debated, especially when a lower FiO_2 is used, for example during laser procedures. As recommended by several authors,[12,21] the routine use of a pulse oximeter is essential.

While hypoxemia was mainly associated with COPD, the occurrence of hypercapnia was dependent on a low preoperative FEV_1. The FEV_1 was reduced both in patients with COPD and in some UAO patients (Fig 3). The impairment of CO_2 elimination may be related to a reduced tidal volume. In COPD patients, this reduction may be explained by increased lower airway resistance, limitation of gas entrainment, and spillage of gas out of the airway. Severe hypercapnia was observed in some patients with UAO (Fig 3), and this alveolar hypoventilation may be explained by limitation of gas entrainment and/or an increased FRC. In fact, an increase in $PaCO_2$ has been reported when FRC was increased by a negative external thoracic pressure during high-frequency ventilation.[22]

Our results suggest that the use of a larger injector may be associated with better gas exchange. An increased insufflated volume (120 mL with the 14-gauge catheter versus 160 mL with the 12-gauge catheter) was responsible for this better alveolar ventilation. As the patients were not randomized for the injector size, it is difficult to recommend an injector diameter or another ventilator setting in patients with airway obstruction. Further comparative studies should be performed to demonstrate an improvement in gas exchange with different ventilator settings according to the clinical condition of the patient (UAO, COPD, or obese).

CONCLUSION

In summary, during laryngoscopy under HFJV, the risk of hyperinflation and barotrauma was increased in UAO, and not in COPD patients. Hypoxemia occurred preferentially in COPD patients, and modified settings or another technique of ventilation should be discussed in such patients, particularly when using low FiO_2. Hypercapnia was seen in both upper and lower obstruction. Detecting HFJV pitfalls is essential. End-expiratory pressure measurement, pulse oximetry, and transtracheal CO_2 monitoring[23] are particularly indicated in obese, COPD, or UAO patients.

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SEVENTH INTERNATIONAL SYMPOSIUM ON THE FACIAL NERVE

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CONGENITAL CONDUCTIVE OR MIXED DEAFNESS, PREAURICULAR SINUS, EXTERNAL EAR ANOMALY, AND COMMISSURAL LIP PITS: AN AUTOSOMAL DOMINANT INHERITED SYNDROME

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Branchiogenic syndromes such as branchio-oto-renal syndrome, Treacher Collins syndrome, and hemifacial microsomia are well delineated. From a clinical study in a large family spanning three generations, it can be concluded that the association of conductive deafness, commissural lip pits, preauricular sinuses, and external ear anomalies can be differentiated from the above-mentioned syndromes and is a separate autosomal dominant syndrome.

KEY WORDS — autosomal dominance, commissural lip pits, congenital hearing loss, ear anomalies, genetic deafness, preauricular sinus.

INTRODUCTION

Congenital deafness is diagnosed in 1 in 1,000 children.[1] Inherited types of deafness are believed to be the cause in almost half of these — thus, in 1 in 2,000.[2] In some of the inherited disorders, hearing loss is the only feature, whereas in others it may be associated with other abnormalities.[3,4] At present, more than 40 autosomal dominant syndromes with congenital hearing loss have been recognized.

An atypical presentation of the branchio-oto-renal (BOR) syndrome (also called ear pit–deafness syndrome) was thought to be the cause of impaired hearing in a father and his twin sons who were referred to the Nijmegen otorhinolaryngology department for hearing evaluation and genetic counseling. The family history revealed additional affected family members, and in consequence, a detailed family study was launched.

METHODS

A genealogical study was performed to complete a pedigree spanning three generations. All 74 living members, plus the spouses of persons II-1 and II-10, were asked to participate in this study. Each person underwent general medical and detailed otorhinolaryngologic assessments. In all persons, any features that were present were recorded by photography.

All persons over the age of 3.5 years were tested audiologically by pure tone audiometry. If this consistently showed conductive or mixed hearing loss, impedance audiometry was also performed.

In four persons (II-10, proband III-7, IV-7, and IV-8) who were suspected of having an atypical presentation of the BOR syndrome, thorough renal assessment had been carried out previously, consisting of blood and urine chemistry, renal ultrasound, and intravenous pyelography. All of these findings were normal. Therefore, we decided to screen the other family members for signs of renal dysfunction using only biochemical methods. However, after 24 of the members were found to be normal, this screening was discontinued.

RESULTS

The family pedigree is presented in Fig 1. All 74 family members, plus the spouses of II-1 and II-10, were examined by the authors. The presence of a preauricular sinus, commissural lip pits, an external ear anomaly, and hearing impairment in the different family members is also shown in the pedigree.

An external ear anomaly, described as lumping and flattening of the superior part of the helix in combination with a reduction in the triangular fossa either unilaterally or bilaterally, was found in 12 persons. Its variability was only minor, as illustrated in Fig 2.

Eleven persons were found to have a unilateral or bilateral preauricular sinus (Fig 1). A preauricular cyst was palpable in II-1 (left) and II-10 (right), which was also derived from the same branchial pouch, whereas there was no evidence of a preauricular sinus or ear pit.

In addition, we found one or more commissural lip pits in eight persons, which had not been noticed previous to the study (Fig 3). These were small sinuses, maximum 4 mm in depth, in the corner of the mouth. Although they can be saliva-productive, they are without clinical importance.

In conclusion, a total of 20 of the 74 persons were

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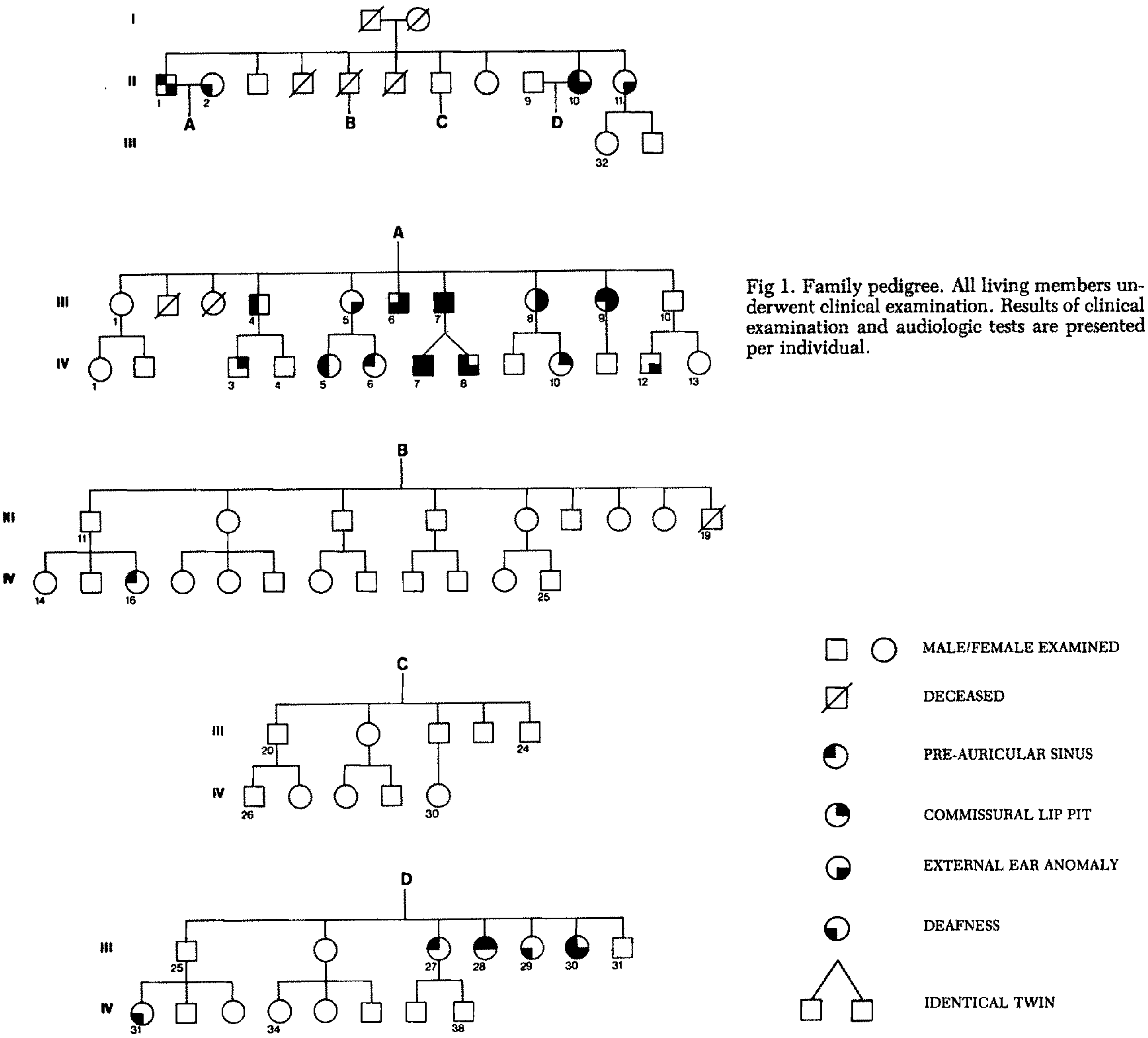

Fig 1. Family pedigree. All living members underwent clinical examination. Results of clinical examination and audiologic tests are presented per individual.

affected by external ear anomalies, preauricular sinuses (or cysts), and commissural lip pits, either in combination or separately, as summarized in Table 1.

General medical examination revealed mild enlargement of the thyroid gland in six female persons (II-10, II-11, III-27, III-29, III-32, and IV-34). A thyroid scan, done in all but one subect (II-11) of the six, confirmed the existence of goiter in only two cases: II-10 and III-32. An iodine uptake and perchlorate study performed in II-10, III-27, III-29, III-32, and IV-34 yielded normal results, so an iodination defect could be excluded.

To differentiate between various branchiogenic syndromes, it is important to mention that we were unable to demonstrate a cervical cyst or cervical branchial arch fistula in any of the 74 family members.

The hearing of 68 persons (spouses II-2 and II-9 inclusive) was assessed by means of pure tone audiometry. All the persons in the remaining group were younger than 3.5 years of age and none of these children were affected by one of the aforementioned anomalies.

Elevated hearing thresholds (defined as a mean threshold >25 dB at frequencies 500, 1,000, and 2,000 Hz) were found in 11 persons (Fig 1 and Table 2). A combination of hearing loss with one of the phenotypic characteristics could be demonstrated in 8 persons. In 6 of them, the hearing loss could only be explained by a hereditary cause (II-10, III-4, III-7, III-30, IV-7, and IV-8). The elevated threshold was mainly conductive, especially in the lower and middle frequencies. Tympanometry, stapedial reflexes, and the operative findings in 3 of them confirmed a presumed middle ear, or more specific, congenital ossicular anomaly, such as stapes ankylosis, and the absence of the long process of the incus. Stapes interpositioning was performed in two

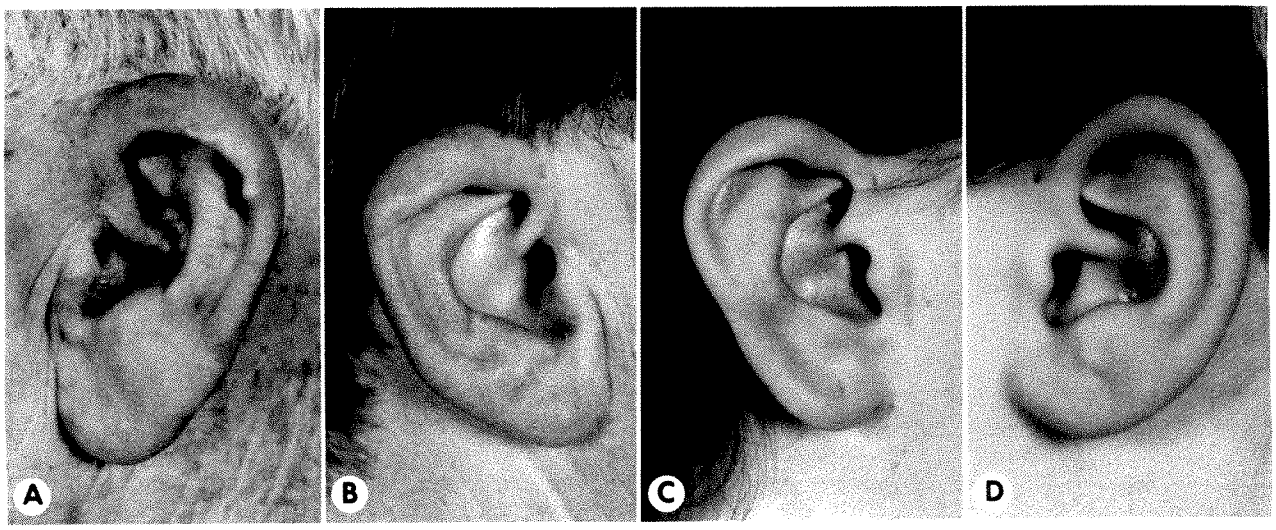

Fig 2. Auricles of A) II-1, B) III-7, C) III-30, and D) IV-6. Three show anomaly of pinna. Preauricular sinus is not associated with external ear anomaly in IV-6.

ears (proband III-7). In one ear (III-30), reconstruction of the ossicular chain with an allograft was carried out. An exploratory tympanotomy was carried out in one ear (II-10) and revealed aplasia of the round window.

Mild progression of the hearing loss could be demonstrated when previous audiograms (performed 1 to 21 years previously) were compared to the present audiograms.

The medical history and otologic examination of the two remaining hearing-impaired persons with phenotypic features (III-6 and IV-5) indicated a hereditary cause for their hearing loss as well, but an acquired cause could not be excluded. Exploratory tympanotomy has not been proposed.

In the two cases of hearing loss without any of the phenotypic features (III-29 and IV-31) and also in II-2, the medical history and otorhinolaryngologic examination confirmed the diagnosis of acquired hearing loss. It is important to note that the progressive mixed deafness in II-2, who is the mother of the proband and the spouse of family member II-1, is very suggestive for otosclerosis. However, the hearing loss of her offspring (III-4, III-6, and III-7) is congenital and only mildly progressive, and stapes ankylosis has been demonstrated.

DISCUSSION

Preauricular sinuses, pinna dysplasia, and deafness can be expressions of branchiogenic disorders as observed in Treacher Collins syndrome, the BOR syndrome, and the hemifacial microsomia syndrome.[5-7] But these features can exist as separate entities as well.

A preauricular sinus or ear pit is found in 1% of the white population, with an autosomal dominant

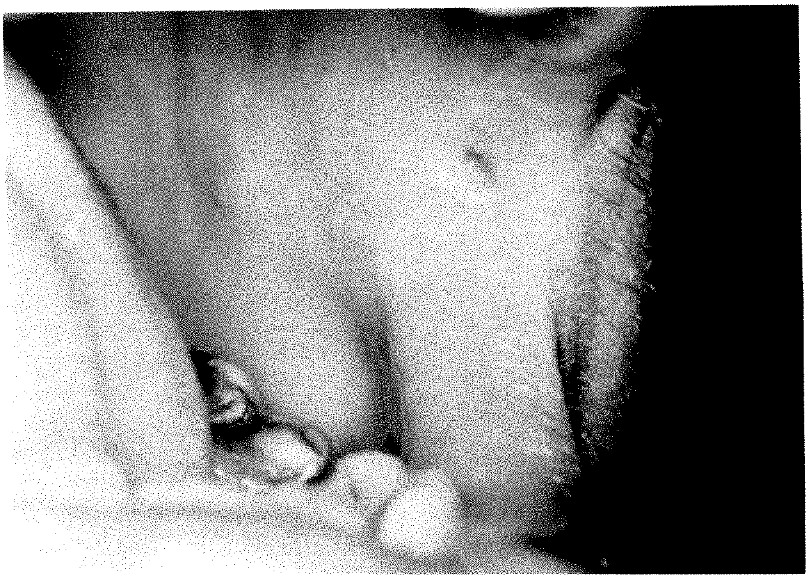

Fig 3. Commissural lip pit in III-28.

TABLE 1. CLINICAL FEATURES OF 20 AFFECTED PERSONS IN THREE GENERATIONS

	Preauricular Sinus		*Commissural Lip Pit*		*External Ear Anomaly*		*Congenital Hearing Loss*	
Code	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
II-1	Cyst	–	–	–	+	+	–	–
II-10	–	Cyst	–	–	+	+	–	+
II-11	–	–	–	–	+/–	+/–	–	–
III-4	+	–	–	–	–	–	–	+
III-5	–	–	–	–	+	+/–	–	–
III-6	–	–	–	+	–	+	+	–
III-7	+	+	+	+	+	+	+	+
III-8	–	–	–	+	–	+	–	–
III-9	+	+	–	+	+	+	–	–
III-27	+	–	–	–	–	–	–	–
III-28	+	+	+	+	–	–	–	–
III-30	–	+	–	–	–	+/–	+	–
IV-3	–	–	+	+	–	–	–	–
IV-5	–	+	–	–	–	–	+	–
IV-6	+	–	–	–	–	–	–	–
IV-7	+	+	+	+	+	+	+	+
IV-8	+	+	–	–	+	+	+	+
IV-10	–	–	–	+	–	–	–	–
IV-12	–	–	–	–	+	+	–	–
IV-16	+	–	–	–	–	–	–	–

Plus — present, minus — absent, plus/minus — subjective.

mode of inheritance.[4,8] Commissural lip pits, contrary to medial lip pits, are said to be even more common; percentages reaching a maximum of 12% have been described.[9,10] Baker[9] demonstrated that there is a significant correlation between the incidence of preauricular sinuses and commissural lip pits: of all the persons with a commissural lip pit, 3.8% also proved to have a preauricular sinus. In our study, a preauricular sinus was found in four of the eight persons with a commissural lip pit. Congenital pinna dysplasia and middle ear malformations are sequelae of disrupted genesis from the six hillocks of the first two branchial arches and the cartilages of Meckel and Reicher, respectively.[11-13] The judgment and interpretation of abnormalities is difficult and often subjective. Nevertheless, in this family, it was thought that the external ear malformation was quite specific.

TABLE 2. PURE TONE THRESHOLDS OF 11 PERSONS WITH HEARING IMPAIRMENT

			Frequencies (Hz)					
Group	*Code*	*Ear*	*250*	*500*	*1,000*	*2,000*	*4,000*	*8,000*
A	II-10	L	20	10+10	0+10	0+10	0+25	30+15
		R	65	10+60	10+60	45+30	30+45	80
	III-4	L	15	10	5	5	15	30
		R	50	5+35	5+35	10+25	0+40	5+40
	III-7	L	40	0+45	5+40	5+30	5+40	0+45
		R	50	0+45	5+40	10+45	10+40	5+40
	III-30	L	50	15+25	10+40	20+25	25+40	75
		R	10	10	10	20	35	50
	IV-7	L	—	60	55	50	45	65
		R	—	10+45	5+55	10+25	0+20	10+20
	IV-8	L	—	40	45	25	25	—
		R	—	0+55	0+45	5+30	0+35	—
B	III-6	L	60	10+40	5+20	10+10	5+35	30+15
		R	30	5+15	5+5	10+0	15+40	40+5
	IV-5	L	60	0+35	0+30	0+25	5+25	40+5
		R	15	5+10	5+15	0+5	5+0	25+15
C	II-2	L	55	50	40	60	75	70
		R	85	85	70	45	55	80
	III-29	L	40	5+45	15+40	25+30	25+35	35+25
		R	55	0+50	0+45	15+30	10+20	15+20
	IV-31	L	—	0+35	0+40	0+30	0+25	—
		R	—	20	30	20	20	—

In pairs of numbers joined by plus symbol, first number is sensorineural component and second number is conductive component.

Group A — six persons with hereditary deafness and phenotypic features as described in Table 1, group B — two persons with possibly acquired deafness and features, group C — two persons with acquired deafness without features and in-law II-2.

The association between congenital conductive or mixed hearing loss and preauricular sinuses, commissural lip pits, and external ear anomalies was so remarkable in this pedigree that it leads us to the conclusion that genetic disease is present. The pattern of inheritance has to be autosomal dominant, because this syndrome proved to be present in three generations and there were male-to-male transmissions as well.

Although several of these features also form part of the BOR syndrome, which is well known to the present authors, we consider that the syndrome reported here is a different entity, mainly because of the absence of cervical fistulae and renal abnormalities and the presence of commissural lip pits.[5,14]

We therefore conclude that the symptoms described in this study represent a congenital syndrome, with an autosomal dominant pattern of inheritance and variable penetrance and expression of the features, that has not been reported previously.

In the future, gene linkage studies are expected to be useful for making a definite differentiation between the various branchiogenic autosomal dominant inherited syndromes.

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AMERICAN RHINOLOGIC SOCIETY RESIDENT'S CASE OF THE YEAR AWARD

The American Rhinologic Society announces the Resident's Case of the Year Award. This contest is open to any resident presenting an interesting case related to rhinology. The deadline is January 1, 1992. Case reports should be sent to: Dr George Facer, Secretary, American Rhinologic Society, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. For more information, call (507) 284-3410.

Ann Otol Rhinol Laryngol 100:1991

GLYCOCONJUGATES IN THE CHINCHILLA TUBOTYMPANUM

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Various biotinylated lectins were used to characterize and semiquantitate glycoconjugate residues in the tubotympanum. Epithelial goblet cells were stained predominantly by WGA, LFA, SNA, RCA-I, Con-A, LCA, SBA, PHA-E, and UEA; this finding suggests they contain α-neuraminic acid, β-galactose, α-mannose, N-acetyl α-galactosamine, and α-fucose. Glandular mucous cells were stained predominantly by WGA, LFA, SNA, and RCA-I; this finding suggests that they contain α-neuraminic acid and β-galactose. The glandular serous cells were stained predominantly by Con-A, WGA, and LFA; this finding suggests that they produced α-mannose and α-neuraminic acid that represented serum-type glycoprotein. The positive staining of epithelial goblet cells and glandular mucous cells with PNA after neuraminidase digestion suggests that they produced mucin-type glycoproteins. The staining of the mucous blanket by WGA, LFA, SNA, RCA-I, LCA, PNA, SBA, PHA-E, and UEA suggests the presence of α-neuraminic acid, β-galactose, N-acetyl α-galactosamine, and α-fucose. The epithelial cell (nonsecretory) surface was stained largely by WGA, LFA, SNA, RCA-I, Con-A, and LCA; this finding suggests the presence of α-neuramimic acid, β-galactose, and α-mannose.

KEY WORDS — chinchilla, eustachian tube, glycoconjugates, lectins, middle ear, neuraminidase.

INTRODUCTION

The study of glycoconjugates has recently become an area of great interest in biomedical research. Glycoconjugates are ubiquitous in animal tissue and are important components of both the cell surface coat and secretory products. Recent histochemical studies of the respiratory and gastrointestinal tracts have focused on the nature of glycoconjugates in secretory cells, particularly mucus-secreting cells, and the identification of glycoconjugates present on epithelial cell surfaces with emphasis on their role in host-parasite interactions.[1-8]

Normal respiratory epithelium is composed of pseudostratified ciliated columnar epithelium and secretory cells, which produce substances involved in mucociliary clearance.[9] Previous histochemical studies have demonstrated glycoconjugates on the epithelial cell surface, in the secretory cells, and in the secretory products of the respiratory epithelium.[1-5,8] The epithelium of the eustachian tube is considered a respiratory epithelium, with approximately 80% being pseudostratified ciliated columnar cells and 20% secretory cells.[10] Glycoconjugates are critical to the secretory products of the mucociliary transport system, which in turn is an important part of the mechanical defense of the middle ear. The main secretory components of the mucociliary transport system include the mucous blanket and the periciliary fluid.[11-13] The mucous blanket is composed of water, solutes of low molecular weight, and a polymer network formed of glycoproteins of high molecular weight, consisting of a protein core with several oligosaccharide side chains.[14] The epithelial goblet cells and glandular mucous cells of the eustachian tube are thought to be the primary secretory cells for the mucous blanket.[10,15] The periciliary serous fluid is believed to be produced locally by serous cells found in the mixed tubuloacinar glands of the eustachian tube.[10,15] Previous work has demonstrated the subsets of the tubotympanal epithelial secretory cells (goblet cells),[16-20] and the dark granulated type were suggested to be serous cells.

Glycoconjugates also play an integral role in the epithelial cell surface and are expressed in the glycocalyx, a polysaccharide-rich layer at the cell surface. The glycocalyx is composed of glycoproteins and glycolipids that are attached to the lipid bilayer of the cell membrane.[21] The glycocalyx of the cell has been demonstrated to play a role in cellular physiology (ie, embryologic development and differentiation)[22] and cellular pathophysiology (ie, host-parasite interaction).[23,24] The adherence of pathogenic bacteria to host cells is one example of host-parasite interaction that may involve glycoconjugates. Indeed, one common mechanism for bacterial-host adherence is the interaction beween bacterial adhesins or fimbriae and specific glycoconjugates present on the epithelial cell surface.[23]

Lectins are carbohydrate-binding proteins of a nonimmune nature that agglutinate cells or precipitate polysaccharides or glycoconjugates.[25] The carbohydrate-binding specificity of lectins is comparable to that of enzymes, and lectins can therefore

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REPRINTS — David J. Lim, MD, Otological Research Laboratories, 4331 University Hospitals Clinic, 456 W 10th Ave, Columbus, OH 43210.

LECTINS	α-Mannose	α-Mannose with α-fucose attached	α-Neu Ac	β-Glc NAc	Neu Ac α-(2,6) Gal/Gal NAc	β-Gal NAc	α-Gal NAc	β-Gal	α-Gal	D-gal β-(1,3)-D-gal NAc	Gal NAc α-(1,3)-Gal NAc	α-Fucose
Wheat germ agglutinin (WGA)			●	●	○							
Succinylated WGA (Succ WGA)				●								
Limax flavus agglutinin (LFA)			●		○							
Sambucus nigra agglutinin (SNA)					●							
Ricinus communis agglutinin (RCA-I)						○		●				
Concanavalin A (Con-A)	●											
Lens culinaris agglutinin (LCA)		●										
Bandeiraea Simplicifolia agglutinin (BSA)							●		●			
Dolichos biflorus agglutinin (DBA)							○				●	
Sophora japonica agglutinin (SJA)						●		○				
Peanut agglutinin (PNA)								●		●		
Soybean agglutinin (SBA)						○	●		○			
Phaseolus vulgaris agglutinin - E (PHA-E)						●	○					
Ulex europaeus agglutinin - I (UEA-I)												●

Fig 1. Lectin carbohydrate specificity. Filled circles — stronger lectin binding, empty circles — weaker lectin binding.

act as a probe to localize different carbohydrate moieties.[25] Through the use of lectins, a detailed analysis can be made of carbohydrate residues present on epithelial cell surfaces and within the secretory products.

This study was undertaken to provide an in-depth characterization of the glycoconjugates found in the secretory cells and in the glycocalyx of middle ear and eustachian tube epithelial cells by the use of lectin labeling. This information could also provide a more complete picture of the biochemical composition of the mucous blanket and periciliary fluid that are essential components of the mucociliary transport system. In addition, this study was intended to elucidate the nature of glycoconjugates on the cell surface that may be involved in the adherence of middle ear pathogens (bacterial and viral) to the tubotympanal mucosa.

MATERIALS AND METHODS

Ten adult chinchillas (400 to 500 g) were used for this study. Following anesthesia with xylazine hydrochloride (1 mg/kg) and ketamine hydrochloride (30 mg/kg), the animal's tissues were fixed by intracardiac perfusion with 10% neutral-buffered formalin and the eustachian tubes were dissected and further fixed for 48 to 72 hours in the same fluid. These specimens were embedded in glycomethacrylate (JB-4 resin, Polyscience, Warrington, Pa) at 4°C and the eustachian tube was divided into pharyngeal, middle, and tympanic portions and reembedded for sectioning. Afterward, the blocks were serial thick sectioned (3 to 4 μm thick) and stained with biotinylated lectins (Vector Laboratories, Burlingame, Calif) by means of the avidin-biotin complex technique similar to that described by Hsu and Raine[26] but modified for glycomethacrylate resin by this laboratory. Briefly, sections were etched in sodium ethoxide (10 minutes); washed in absolute alcohol; hydrated in 95% ethanol, 80% ethanol, and 70% ethanol; and rinsed in water. Slides were then exposed to 3% hydrogen peroxide in absolute methanol (20 minutes), washed in tap water (10 minutes), rinsed in distilled water, and placed in Tris-buffered saline (pH 7.4). Sections were then incubated in mouse liver powder solution (0.1 mg/100 mL) at 37°C (20 minutes) to block nonspecific lectin binding, covered with biotinylated lectins at 4°C (24 hours), and washed two times with Tris-buffered saline (10 minutes each). Slides were incubated with avidin-biotin complex at 37°C (60 minutes), washed, transferred to acetate buffer (0.02 mol/L, pH 5.2, 5 minutes), and then immersed in 3-amino-9-ethylcarbazole peroxidase (10 minutes). Sections were rinsed twice in acetate buffer (0.02 mol/L, pH 5.2, 5 minutes), rinsed in hot water (10 minutes), counterstained with Mayer's hematoxylin (15 to 20 minutes), dipped in ammonia water (0.1%; five to nine times), and then washed in hot water (10 minutes). Coverslips were applied with an aqueous mounting medium (glycerin jelly) and slides were examined by light microscopy.

Lectins selected were wheat germ agglutinin (WGA; specific for α-neuraminic acid [Neu Ac], *N*-acetyl β-glucosamine [β-Glc NAc]), Succ WGA

LECTIN	Pharyngeal Portion			Mid Portion			Tympanic Portion
	Epithelial Goblet	Glandular mucous	Glandular serous	Epithelial Goblet	Glandular mucous	Glandular serous	Epithelial Goblet
WGA	2+/86%	2+/47%	1+/50%	3+/81% / 1+/19%	3+/100%	1+/50%	3+/82% / 1+/18%
Succ WGA	1+/20%	0	0	1+/2%	0	0	0
LFA	2+/75%	3+/100%	1+/50%	2+/87%	3+/100%	1+/80%	3+/80%
SNA	3+/85% / 1+/15%	3+/100%	0	3+/88% / 1+/12%	3+/100%	0	3+/83% / 1+/17%
RCA-I	3+/81%	3+/100%	1+/50%	3+/88% / 1+/12%	3+/100%	0	3+/82% / 1+/18%
Con-A	1+/73%	2+/100%	3+/100%	1+/6%	0	3+/75%	3+/84% / 1+/16%
LCA	1+/46%	1+/27%	0	2+/77%	1+/50%	0	3+/77%
BSA	2+/34%	2+/19%	0	2+/17%	2+/7%	0	0
DBA	1+/11%	3+/28%	1+/53%	3+/3%	3+/2%	0	0
SJA	1+/19%	1+/1%	0	0	0	0	2+/82%
PNA	2+/25%	2+/5%	0	2+/22%	3+/2%	0	3+/50%
PNA*	2+/40% / 1+20%	2+/80%	0	2+/45% / 1+25%	2+/75%	0	3+/90%
SBA	3+/81%	3+/12%	1+/18%	3+/95%	3+/4%	0	3+/67%
PHA-E	2+/71%	1+/11%	1+/94%	2+/35%	1+/2%	0	2+/81%
UEA	1+/77%	1+/37%	0	3+/63%	1+/41%	0	2+/22%

Fig 2. Lectin labeling of secretory cells of eustachian tube. 1+ — weak labeling, 2+ — moderate labeling, 3+ — strong labeling, asterisk — after neuraminidase digestion.

(specific for β-Glc NAc), LFA (specific for α-neuraminic acid), SNA (specific for Neu Ac [α2,6] Gal/Gal NAc), RCA-I (specific for β-galactose [β-Gal] and *N*-acetyl-β-galactosamine [β-Gal NAc]), concanavalin A (Con-A; specific for α-mannose), LCA (specific for α-mannose with α-fucose), BSA (specific for α-galactose and α-Gal NAc), DBA (specific for Gal NAc [α-1,3] Gal NAc), SJA (specific for β-galactose and β-Gal NAc), peanut agglutinin (PNA; specific for β-galactose, especially Gal [β1,3] Gal NAc), soybean agglutinin (SBA; specific for α-Gal NAc, especially Gal NAc [α1,3] Gal), PHA-E (specific for α,β-Gal NAc), and UEA (specific for α-fucose)[8,25] (Fig 1). Neuraminic acids (sialic acids) were removed by incubating sections in a solution of neuraminidase (type V, Sigma, St Louis, Mo) 0.2 U/mL in acetate buffer, pH 5.5, at 37°C for 3 hours before staining with PNA.

For control, sections were incubated with mouse liver powder solution, following avidin-biotin complex, to block nonspecific labeling.

Results were semiquantified by using a video image analyzer with a computerized morphometric system (Bioquant II). Intensity of the lectin staining was arbitrarily categorized as weak (1+), moderate (2+), or strong (3+). The percentages of the labeled cells for the different areas were determined by counting total cells per microscopic field using a digitizer, with at least three slides used to determine an average percentage.

RESULTS

The lectin labeling patterns for the different portions of the eustachian tube were essentially the same across animals.

Epithelial Goblet Cells. Although there were subtle differences of lectin labeling among different portions (pharyngeal, middle, and tympanic) of the eustachian tube, the general pattern of lectin labeling of different parts of the tubotympanum was similar (Figs 2 and 3). The epithelial goblet cells demonstrated moderate to strong labeling of discrete secretory granules by WGA (Fig 4A), RCA-I (Fig 4B), LFA (Fig 5A), SNA (Fig 5B), SBA, PNA, Con-A (tympanic portion), LCA (middle and tympanic portions), DBA (middle portion), SJA (tympanic portion), and PHA-E. However, the labeling with UEA was strong in the middle portion and moderate in the tympanic portion, but weak in the pharyngeal portion of the tube. Weak labeling of the secretory granules was seen with Succ WGA, Con-A (middle and pharyngeal portions), LCA (pharyngeal portion), DBA (pharyngeal portion), and SJA (pharyngeal portion). After neuraminidase treatment, more than 50% of the epithelial goblet cells became moderately to strongly labeled with PNA (Fig 6).

The following lectins labeled the majority of epithelial goblet cells and are listed in order of decreasing intensity of labeling: SNA, RCA-I, SBA, WGA, LFA, LCA, Con-A, PHA-E, and UEA (Fig 2). Also of note is that certain lectins, including WGA, SNA, RCA-I, and Con-A, labeled two distinct subsets of epithelial goblet cells differently. While a large number of epithelial goblet cells was labeled strongly by these lectins, a small number of this cell

LECTIN	Labeling Pattern of the Cell Surface of the Eustachian Tube			Labeling Pattern of the Mucous Blanket
	Pharyngeal Portion	Mid Portion	Tympanic Portion	Mucous Blanket
WGA	3+ Entire	2+ Entire	3+ Entire	2+ Entire
Succ WGA	0	2+ Roof, Neck, mid	1+ Roof, Neck, mid	1+ Entire
LFA	2+ Entire	2+ Entire	3+ Entire	2+ Entire
SNA	3+ Entire	3+ Entire	3+ Entire	3+ Entire
RCA-I	3+ Entire	3+ Entire	3+ Entire	3+ Entire
Con-A	3+ Entire	2+ Roof, Neck, mid	2+ Entire	1+ Partial
LCA	0	3+ Roof, Neck, mid	1+ Entire	3+ Entire
BSA	0	1+ Neck	0	1+ Partial
DBA	0	3+ Neck	0	1+ Partial
SJA	0	0	0	1+ Partial
PNA	0	3+ Neck	1+ Entire	2+ Entire
PNA*	0	3+ Neck	1+ Entire	3+ Entire
SBA	0	2+ Roof, Neck, mid	3+ Roof, Neck mid	3+ Entire
PHA-E	0	0	0	2+ Entire
UEA	0	0	0	2+ Entire

Fig 3. Lectin labeling of epithelial cell surface and mucous blanket of eustachian tube. 1+ — weak labeling, 2+ — moderate labeling, 3+ — strong labeling, asterisk — after neuraminidase digestion.

population was weakly labeled by the same lectins. For example, far less than 50% of the epithelial goblet cells were labeled by PNA, DBA, BSA, and Succ WGA (Fig 2).

Some lectins demonstrated regional differences in the labeling pattern of the epithelial goblet cells; for example, Succ WGA, BSA, and DBA labeled a larger percentage of cells in the pharyngeal portion, followed by the middle portion, and did not label any goblet cells in the tympanic portion (Fig 2). It is important to note that SBA, on the other hand, labeled a majority (67%) of goblet cells in the tympanic portion (Fig 2). Con-A and PHA-E labeled a majority of epithelial goblet cells in the pharyngeal and tympanic portions, but only a minority of these cells in the middle portion. UEA labeled a majority of epithelial goblet cells in the pharyngeal and middle portions but only a minority of these cells in the tympanic portion. LCA stained a majority of epithelial goblet cells in the middle and tympanic portions but only a minority of these cells in the pharyngeal portion. SJA, however, labeled a majority of epithelial goblet cells in the tympanic portion, followed by the pharyngeal portion, and did not label epithelial goblet cells in the middle portion (Fig 2).

Glandular Mucous Cells. The glandular mucous cells demonstrated lectin labeling of discrete secretory granules. Moderate to strong labeling of these granules was seen with RCA-I (Fig 7A), LFA (Fig 7B), SNA (Fig 7C), WGA (Fig 7D), SBA, and DBA. From weak to moderate labeling of these granules was seen with PNA, Con-A, LCA, PHA-E, and UEA (Fig 2). Almost 80% of glandular mucous cells labeled moderately to strongly with PNA after neuraminidase treatment (Figs 2 and 8).

The following lectins labeled the majority of glandular mucous cells and are listed in order of decreasing intensity of labeling and percentage of cells labeled: RCA-I, LFA, SNA, WGA, and Con-A. Less than 50% of the glandular mucous cells were labeled by LCA, UEA, PHA-E, SBA, PNA, SJA, DBA, BSA, and Succ WGA (Fig 2).

Some lectins demonstrated regional differences in labeling of glandular mucous cells of the eustachian tube. For example, Con-A labeled a majority of glandular mucous cells in the pharyngeal portion, but labeled none of these cells in the middle portion. DBA labeled a larger percentage of glandular mucous cells in the pharyngeal portion than in the middle portion, whereas LCA labeled a larger percentage of glandular mucous cells in the middle portion than in the pharyngeal portion (Fig 2).

Glandular Serous Cells. The glandular serous cells demonstrated diffuse labeling of their cytoplasm. These cells were strongly labeled by Con-A (Fig 9) and were weakly labeled by WGA, LFA (Fig 7B), DBA, SBA, and PHA-E (Figs 2 and 3).

A majority of the glandular serous cells were labeled by Con-A, WGA, and LFA. Of the remaining lectins tested, only DBA, SBA, PHA-E, and RCA-I exhibited any labeling of these cells. Neuraminidase treatment had no influence on PNA labeling of glandular serous cells (Fig 2).

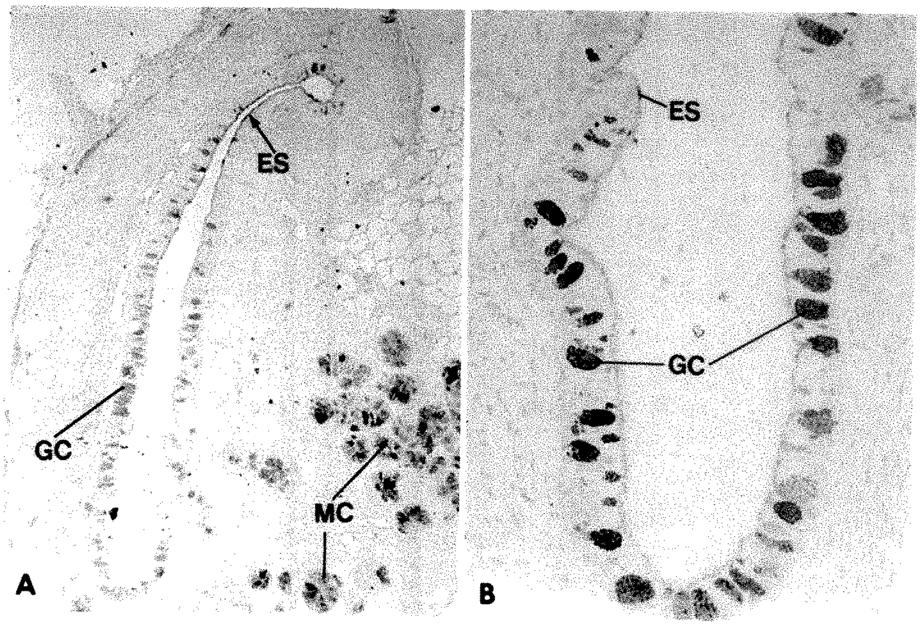

Fig 4. Moderate to strong labeling of glandular mucous cells (MC), epithelial goblet cells (GC), or epithelial cell surface (ES) of middle portion of eustachian tube with A) WGA (original x50) or B) RCA-I (original x160).

Regional differences in lectin staining of the glandular serous cells were also observed. Certain lectins such as RCA-I, DBA, SBA, and PHA-E labeled glandular serous cells in the pharyngeal portion, but did not label these cells in the middle portion (Fig 2).

Mucous Blanket. Moderate to strong labeling of the mucous blanket was seen with RCA-I, LCA, SBA, LFA (Fig 5A), SNA (Fig 5B), PNA, PHA-E, and UEA (Fig 3). Also of interest was the fact that even lectins that labeled the entire mucous blanket did so in a nonuniform fashion: certain areas of the mucous blanket directly apposed to discharging epithelial goblet cells were very darkly labeled, while adjacent areas of the mucous blanket were very lightly labeled (Fig 5A). After neuraminidase treatment, PNA strongly labeled the mucous blanket (Fig 3).

Cell Surface Coat. Moderate to strong labeling of the cell surface coat (particularly the cilia) was seen with WGA (Fig 10A,B), SNA (Fig 5B), RCA-I, Con-A (Fig 10C,D), LFA (Fig 10E,F), SBA, and LCA (middle portion). Weak labeling was seen with Succ WGA, LCA (tympanic portion), BSA, DBA, and PNA.

The following lectins labeled the entire epithelial cell surface of the tubal lumen: WGA, RCA-I, SNA, LFA, and Con-A. In contrast to these lectins, LCA, Succ WGA, BSA, DBA, PNA, and SBA labeled only a portion of the epithelial cell surface of the tubal lumen (Fig 3).

Regional differences in lectin labeling of the epithelial cell surface were also observed. Certain lectins such as Succ WGA, LCA, PNA, and SBA labeled the epithelial cell surface of the middle and

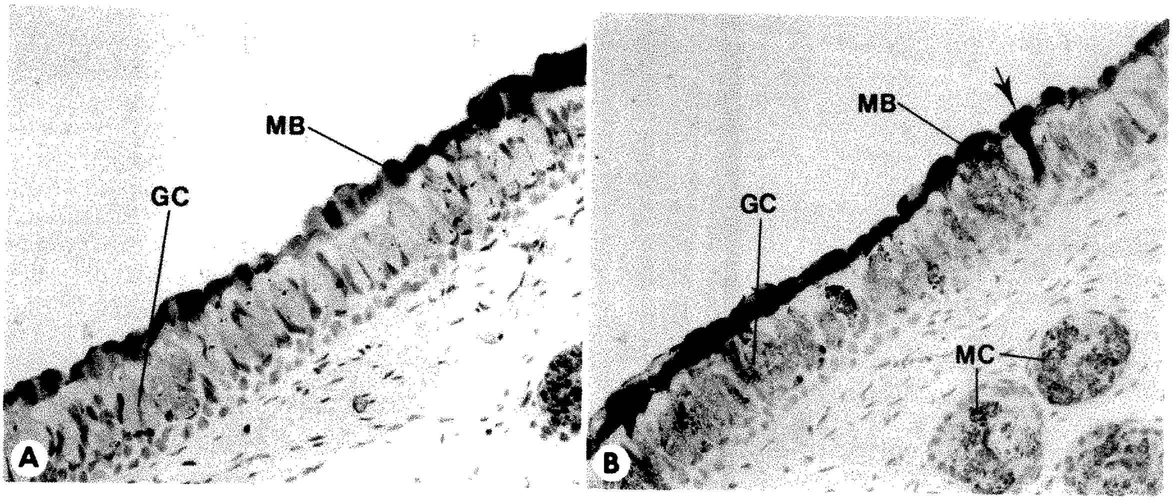

Fig 5. Original x250. Lectin labeling of mucous blanket (MB), goblet cells (GC), and glandular mucous cells (MC) of pharyngeal portion of eustachian tube. A) Uneven labeling with LFA appears to reflect secretory products (bolus) of underlying GC, which are moderately labeled. B) Moderate to strong labeling with SNA. Arrow — GC in process of secretion of MB.

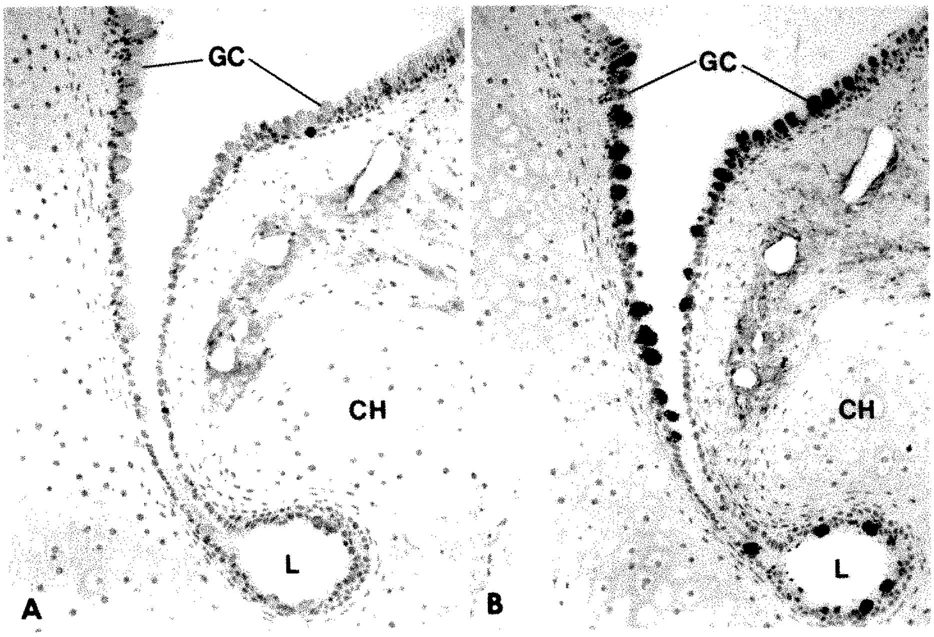

Fig 6. Tympanic portion of eustachian tube (cross section) labeled with PNA before and after neuraminidase treatment (original x50). L — tubal lumen, CH — chondrocytes. A) Before treatment only 50% of goblet cells (GC) moderately label. B) After treatment, 90% strongly label.

tympanic portions, but did not label the epithelial cell surface of the pharyngeal portion. In contrast, other lectins, including BSA and DBA, labeled only a small area of the epithelial surface of the tubal lumen of the middle portion, but did not label the epithelial surface of the pharyngeal or tympanic portions (Fig 3).

Control. There was no labeling when the sections were incubated with mouse liver powder solution.

DISCUSSION

Previous studies have identified three essential prerequisites for the establishment of otitis media: 1) bacterial adherence to the nasopharynx, 2) bacterial entry into the tubotympanum, and 3) bacterial replication in the tubotympanum.[27] Understanding this complex host-parasite interaction requires in-depth knowledge of the biochemical composition of the secretory products and the epithelial cell surface coat that interact with invading organisms. Glycoconjugates are ubiquitous in the secretory cells of the tubotympanum and are thought to be integral components of the mucociliary transport system that serves as a mechanical defense against entry of pathogenic bacteria into the middle ear. In addition, glycoconjugates located in the glycocalyx overlying the epithelial cells play an important role in cellular pathophysiology by serving as potential receptors for pathogenic bacteria.[23] The present findings are consistent with previous studies from this laboratory, which have also demonstrated heterogeneity of secretory cells in the tubotympanum. Three secretory cell types can be identified based on the presence of osmiophilic granules[16,17]: light granules, primarily located in the epithelial goblet and glandular mucous cells; dark granules, found primarily in the serous demilunes and cuboidal secretory cells of the transitional zone; and intermediate or mixed granules.[16-18,28] The light-granulated cells are thought to secrete mucus and have been found to contain acidic and neutral mucosubstances,[16] incorporate a large amount of radiolabeled glucose, and produce lysozyme,[20,29] whereas the dark-granulated cells are thought to produce serous secretions, do not demonstrate acidic or neutral mucosubstances,[16] incorporate a large amount of radiolabeled leucine,[19] and produce lactoferrin.[20,29]

These examples of biochemical heterogeneity may indicate that mucosal secretions play a dynamic functional role in maintenance of the normal physiology of a ciliated epithelium. The ciliated mucosal epithelium is composed of a mucous blanket overlying beating ciliated cells that are bathed in periciliary fluids. Even within the mucous blanket, a carefully regulated mechanism exists to ensure the optimal viscoelasticity of mucus, which in turn maximizes effectiveness of ciliary beating.[30] In a fully functional mucociliary transport system, cilia beat within the sol or periciliary fluid layer and the tips of the cilia penetrate the mucous blanket to propel it forward.[11,12,30,31] The complexity of this interaction dictates that both the nature and amount of mucous blanket secretion and periciliary secretion be maintained within tight parameters.[32] The rheologic properties of the mucous blanket are primarily attributable to the glycoprotein composition of the secretory products.[33] The complexity of the secretory products noted in the present study is in line with these earlier studies.

Glycoproteins can be classified by the nature of the linkage between the oligosaccharide chains and the polypeptide. The oligosaccharide chains are heterogeneous with respect to sugar compositions. It was reported that bronchotransferrin and secretory immunoglobulin A belong to the "serum"-type

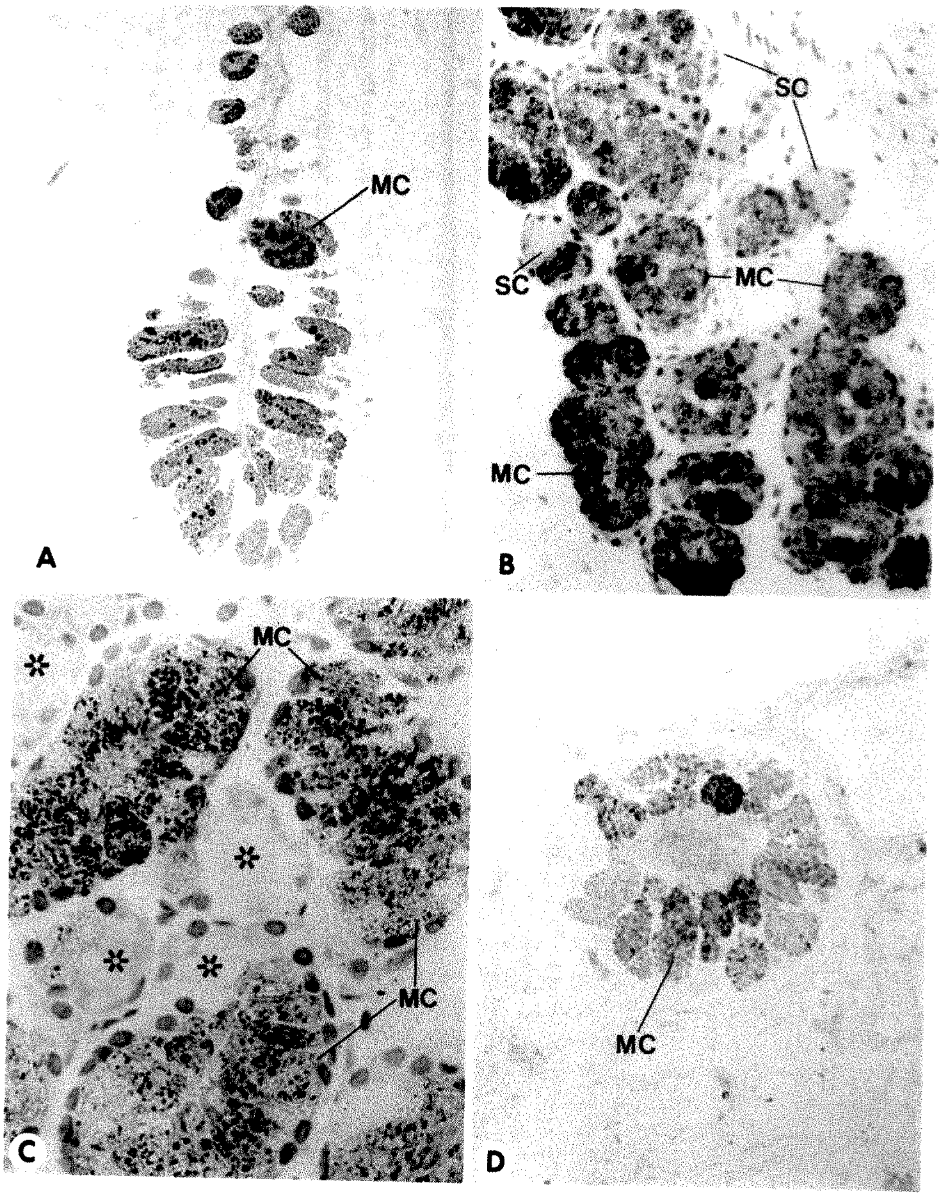

Fig 7. Glandular mucous cells (MC) of middle portion of eustachian tube. A) Strongly labeled with RCA-I (original x250). B) Strongly labeled with LFA (original x400). Glandular serous cells (SC) are weakly labeled with LFA. C) Glandular MC of middle portion are strongly labeled with SNA (original x400). Glandular SC (asterisks) are not labeled with SNA. D) Glandular SC are strongly labeled with WGA (original x400).

glycoproteins, and bronchial mucous glycoproteins belong to the "mucin"-type glycoproteins.[34] Some lectins may specifically recognize "mucin" or "serum"-type carbohydrate side chains linked to the protein cores of glycoprotein.[35] Side chains of the mucin type are reported to be recognized by PNA, while the serum type may be preferentially recognized by Con-A. Wheat germ agglutinin and SBA are thought to react with both types.[35] The present results indicated that the tubotympanum possessed both mucin and serum types of glycoprotein. It is also important to note that sialidase digestion was needed to reveal the presence of this mucin type when labeled with PNA.

By the use of biotinylated lectins, we have identified the major carbohydrate moieties expressed in the glycocalyx of the eustachian tube epithelium to be those labeled by WGA, LFA, SNA, RCA-I, Con-A, and LCA. The characterization of these residues is significant because many of the carbohydrate residues (neuraminic acid [sialic acid], galactose, mannose, and fucose) to which they bind are known to be components of receptors involved in bacterial adherence.[23] Previous investigators have demonstrated that the availability of specific carbohydrates found on host tissue may influence the ability of pathogenic organisms to initiate infection.[23] Indeed, many studies have shown a decreased incidence of infection by certain pathogens after blocking of specific carbohydrate residues located in the glycocalyx of epithelial cells.[36-38]

Using lectin histochemistry, we have successfully identified important carbohydrate constituents of different secretory cells in the tubotympanum. Data indicated that the main components of the epithelial goblet cells were sialic acid, sialic acid (α2,6) Gal/Gal NAc, β-galactose, α-mannose, α-mannose with α-fucose, α-Gal NAc, β-Gal NAc, and α-fu-

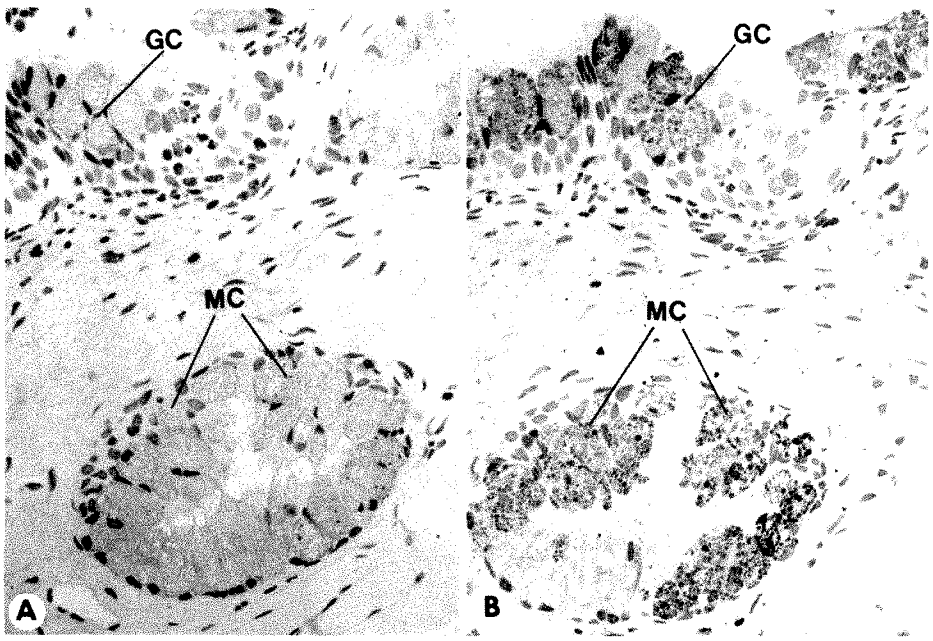

Fig 8. Pharyngeal portion of eustachian tube (cross section) labeled with PNA before and after neuraminidase digestion (original x400). A) Before digestion, epithelial goblet cells (GC) and glandular mucous cells (MC) weakly label. B) After digestion there is moderate labeling of these cell types.

cose. Because of the labeling patterns of a small subset of epithelial goblet cells, this subset was thought to represent the intermediate or dark-granulated cells previously described by Hussl and Lim,[16] Lim and Hussl,[17] and Lim.[18] The main components of the glandular mucous cells were interpreted to be sialic acid, sialic acid (α2,6) Gal/Gal NAc, and β-galactose. These findings are consistent with previous investigations that indicate that RCA-I and WGA stain mucous secretory cells in the human bronchus.[39]

In contrast to the consistent labeling of epithelial goblet cells and glandular mucous cells with WGA, SNA, RCA-I, LFA, and Con-A, marked variability in labeling was noted within these two cell populations when they were labeled with 1) PNA, DBA, BSA, and Succ WGA and 2) UEA, PHA-E, SBA, PNA, SJA, DBA, BSA, and Succ WGA, respectively. This individual variability of reactions to these lectins is interpreted to reflect their biochemical heterogeneity. This biochemical heterogeneity of carbohydrate secretions is consistent with previous studies that demonstrated a similar phenomenon in goblet cells of the rat colon[1] and in bronchial goblet cells.[8] The heterogeneity noted in rat colon goblet cells was suggested to be due to dynamic differences in cellular synthesis or degradation, or to cellular differentiation.[1]

The lectin labeling pattern of the mucous blanket indicated that the main carbohydrate components were sialic acid, sialic acid (α2,6) Gal/Gal NAc, β-galactose, α-mannose with α-fucose, α-Gal NAc, and α-fucose. These results indicate that the mucous blanket is biochemically heterogeneous with regard to carbohydrate composition. By comparing

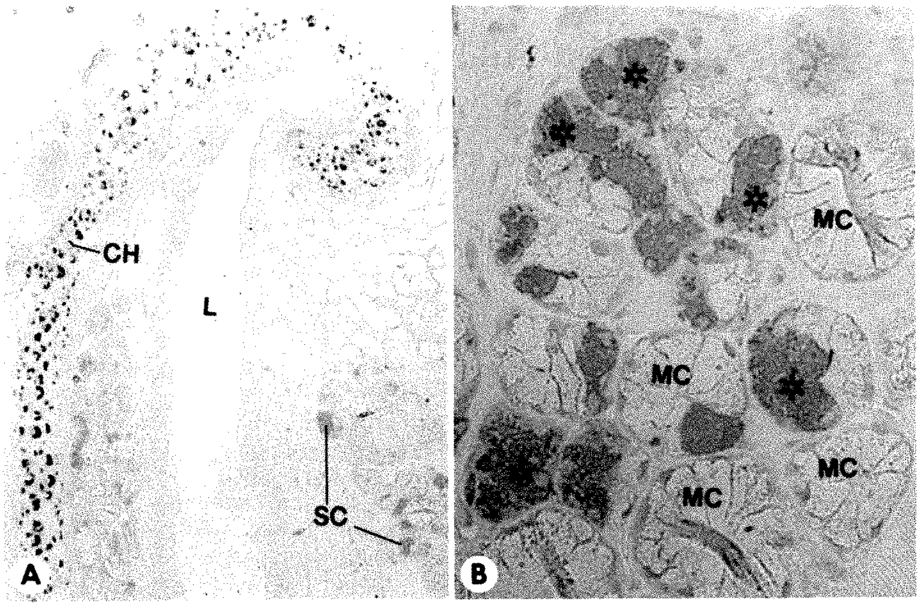

Fig 9. Glandular serous cells (SC) and chondrocytes (CH) of middle portion of eustachian tube are strongly labeled with Con A. A) Original x50. L — tubal lumen. B) Higher magnification of tubal glands shows positive labeling of glandular SC (asterisks; original x150). Glandular mucous cells (MC) are not labeled.

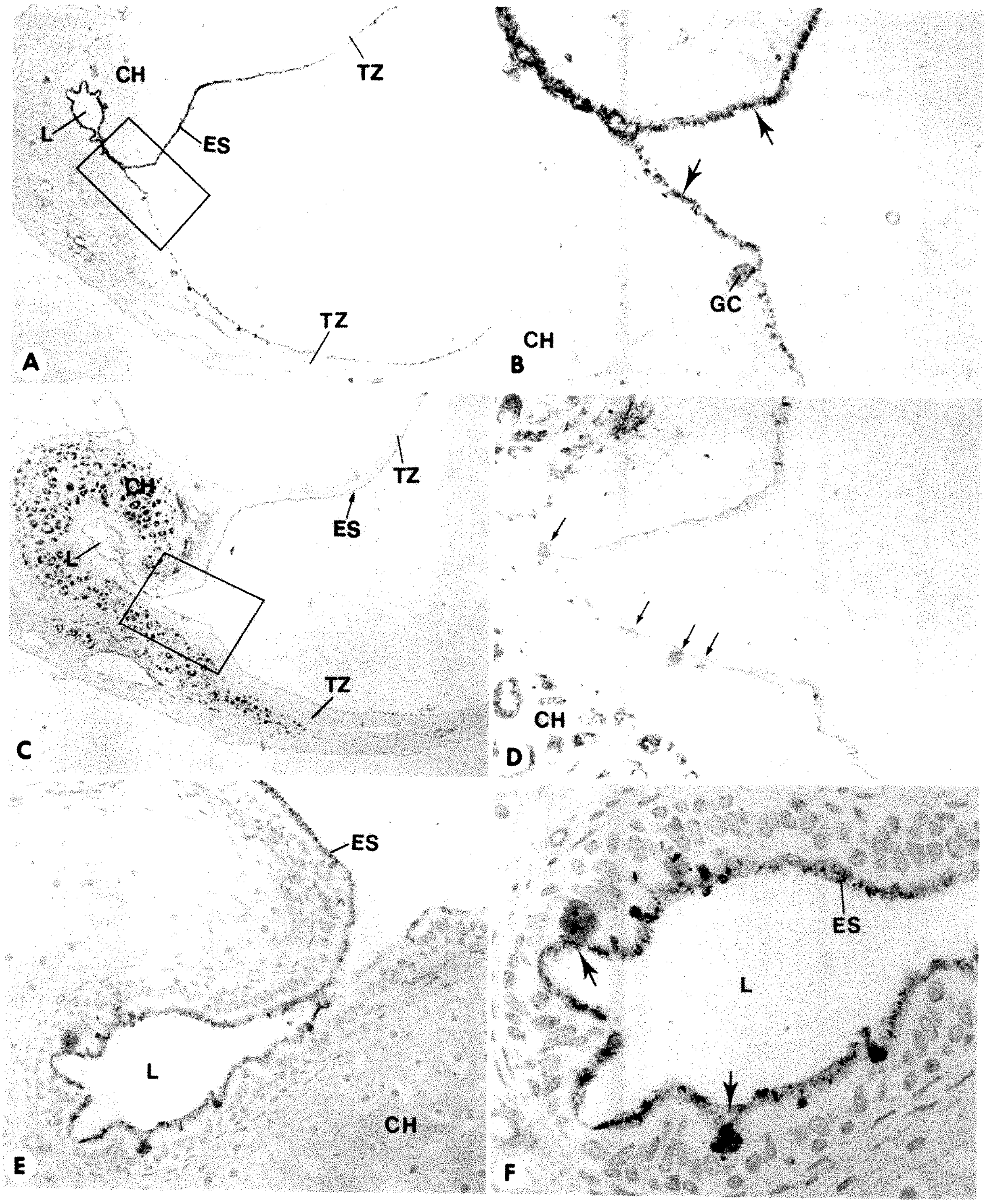

Fig 10. Low (A,C,E) and higher (B,D,F) magnifications of tympanic portion of eustachian tube labeled with lectins. L — tubal lumen, CH — chondrocytes, TZ — transitional zone. A) WGA labeling of epithelial cell surface (ES) is strong (original x50). B) Enlargement of rectangular area from A (original x250). CH are not labeled. Arrows — ES, GC — epithelial goblet cell. C) Con A labeling of ES is moderate, whereas CH of tubal cartilage strongly label (original x50). D) Enlargement of rectangular area from C (original x250). Moderate labeling of ES and GC (arrows) and strong labeling of CH. E) Moderate LFA labeling of ES (original x250). F) Close-up view of E shows moderate labeling of GC (arrows) and strong labeling of ES (original x400).

the lectin staining pattern of the mucous blanket with secretory cells in the tubotympanum, we find that the epithelial goblet cells demonstrate a carbohydrate profile that is similar to that found in the mucous blanket in the immediate vicinity. These results may be interpreted to indicate that the epithelial goblet cells are largely responsible for mucous blanket production, but that there are subsets of goblet cell populations that contribute different biochemical components of the mucous blanket.

The composition of the periciliary fluid is equally significant to the maintenance of middle ear homeostasis. The periciliary fluid or sol is the milieu in which cilia make their recovery stroke and in which all epithelial products are initially released. An inadequate volume of periciliary fluid would result in excessive penetration of the ciliary tip and shaft into the mucus, thus inhibiting their beating. Such situations were observed in the case of chronic otitis media with effusion or serous otitis media[40,41] and in the ear with experimental immune complex–mediated otitis media[42] or influenza A–induced otitis media (Ohashi et al, unpublished data). Conversely, an excessive sol layer would not permit coupling with the overlying mucous blanket. The glandular serous cells produce secretions that are thought to contribute to the periciliary fluid. In the present study, these cells did not demonstrate the same degree of biochemical heterogeneity as seen with the epithelial goblet cells and the glandular mucous cells. These cells were stained predominantly by Con-A, WGA, and LFA. Therefore, α-mannose and α-neuraminic acid were found to be the major lectin-binding carbohydrate secretory products. These findings are consistent with other studies that have examined serous cells in the respiratory and gastrointestinal tracts.[35,43,44] In the present study, we have demonstrated that a subset of epithelial goblet cells (or serous cells) also produce serous fluid.

Clearly, the secretions of the middle ear epithelium are not simply lubricants. It is well documented that diseases such as cystic fibrosis that result in mucus of abnormal glycoconjugate composition and altered rheologic properties[45] predispose to a vast array of respiratory disease and dysfunction.[46,47] Mucoid otitis media, which is characterized by a highly viscous middle ear fluid, is another example of an overly sticky secretion that is too tenacious to be removed by the ciliary apparatus of the middle ear,[19] although in this example it has not yet been determined if the altered rheologic properties are a result of presecretion or postsecretion dysfunction. We must therefore understand these secretions better if we are to attempt to unravel the molecular process involved in otitis media with effusion.

This study provides a normal baseline for the distribution of specific carbohydrate components of the secretory glands and cell surface coat of the tubotympanum. These glycoconjugates have broad biologic functions such as playing a protective role in the normal mucociliary defense mechanism and in participating in adherence of pathogenic organisms that may lead to otitis media.

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AFFERENT ACTIVITY IN THE EXTERNAL BRANCH OF THE SUPERIOR LARYNGEAL AND RECURRENT LARYNGEAL NERVES

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We investigated the presence of respiratory-modulated receptors in the recurrent laryngeal nerve (RLN) and the external branch of the superior laryngeal nerve (ExtSLN) in anesthetized, spontaneously breathing dogs. Of 39 receptors recorded from the ExtSLN, the vast majority responded with a slowly adapting discharge to compression of the cricothyroid muscle, and only 1 responded to probing of the laryngeal mucosa. Ten receptors showed a respiratory modulation. All 30 receptors recorded from the RLN responded to probing of the laryngeal lumen, most of them (60%) with a rapidly adapting response. Seven of the slowly adapting receptors exhibited a respiratory modulation; 38% of the receptors tested were stimulated by water, and only 15% by smoke. No receptors stimulated by laryngeal cooling were identified in either nerve. Our study indicates that in the RLN and the ExtSLN there are relatively few afferents responding to changes in transmural pressure and mechanical irritation, as compared to the internal branch of the SLN. The relative scarcity of receptors responding to transmural pressure and irritant stimuli is consistent with previous observations in dogs that indicate a preponderant role for afferents in the internal branch of the SLN in the reflex responses to laryngeal stimulation.

KEY WORDS — laryngeal afferents, larynx, recurrent laryngeal nerve, superior laryngeal nerve.

INTRODUCTION

Interest in studying the afferent activity emerging from the larynx stems from the well-recognized role of this portion of the airway in defensive[1,2] and respiratory reflexes. The latter responses, related to transmural pressure and airflow, modify the pattern of breathing and the activity of upper airway muscles.[3-6] In addition, laryngeal afferents may be involved in the regulation of airway smooth muscle tone, since several types of laryngeal receptors have been shown to be affected by cooling,[7-10] and upper airway cooling induces bronchoconstriction.[11]

The available evidence indicates that the afferent innervation to the larynx is provided by the internal branch of the superior laryngeal nerve (IntSLN) for the supraglottic region and by the recurrent laryngeal nerve (RLN) and the external branch of the superior laryngeal nerve (ExtSLN) for the subglottic portion.[7,12-14] Since the RLN and the ExtSLN supply motor fibers to the intrinsic muscles of the larynx, they are expected, as in the case of other muscle nerves, to provide also the proprioceptive innervation. Fiber diameter spectra of the RLN are consistent with a proprioceptive component.[15] It must be noted, however, that a proprioceptive innervation of laryngeal muscles has been both denied[16] and affirmed.[14,17] The contribution to the control of breathing and defensive responses of afferents traveling in the RLN and ExtSLN has generally been considered as marginal. Although several studies have described responses to pressure, cold, and irritant stimuli that were virtually eliminated by superior laryngeal nerve (SLN) section,[4-6,11] a possible contribution of RLN afferents cannot be entirely excluded. These afferents could in fact provide some subliminal input to which IntSLN afferents could add their activity with the result of a greater overall response. Moreover, it has long been known that electrical stimulation of the RLN central cut end can alter respiratory and cardiovascular functions.[18,19]

The aim of this study is to identify RLN and ExtSLN receptors responsive to changes in pressure across the laryngeal wall (transmural pressure), temperature, and mechanical and chemical irritant stimuli.

METHODS

Experiments were performed on 17 mongrel dogs (11 females and 6 males), weighing between 10 and 15 kg, anesthetized with a mixture of α-chloralose (0.1 g/kg) and urethane (1.0 g/kg) injected intravenously. The dogs were placed on an operating table in the supine position. A femoral vein was cannulated for further injections of the anesthetic mixture. Arterial blood pressure was monitored by connecting a pressure transducer to a polyethylene catheter inserted into a femoral artery. Anesthesia was maintained at a level adequate to eliminate pain during all surgical and experimental procedures, in accordance with *Guide for the Care and Use of Laboratory Animals* (DHEW Publication No. 78-23).

From the Departments of Physiology and Biophysics (F. Sant'Ambrogio, Tsubone, G. Sant'Ambrogio) and Pediatrics (Mathew), The University of Texas Medical Branch at Galveston, Galveston, Texas. Supported by National Institutes of Health grants HL-20122 and HL-32921. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC protocol 89-09-203) of The University of Texas Medical Branch at Galveston.

REPRINTS — Franca B. Sant'Ambrogio, PhD, DNSc, Dept of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, TX 77550-2781.

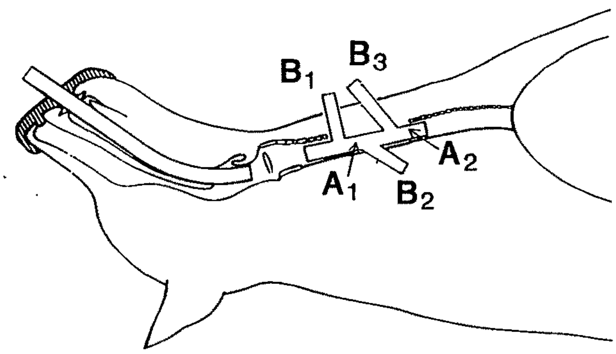

Fig 1. Schema of experimental setup. Face mask made with quick-setting epoxy is built around oral cannula, sealing mouth and nares. Tracheal cannula with three sidearms allowed diversion of breathing from tracheostomy (oral cannula, sidearms B1 and B2 occluded) to upper airway (oral cannula open, sidearms B1 through B3 occluded). Tracheal occlusion was performed by inflating cuff of Foley catheter at A2; upper airway occlusion, by occluding oral cannula as well as three sidearms of tracheal cannula. Isolation of upper airway was accomplished by inflating cuff of Foley catheter at A1.

The cervical trachea was exposed in its entire length, and the ventral aspect of four to five cartilaginous rings was cut longitudinally to allow the introduction of both ends of a cannula having three sidearms.

A saline-filled polyethylene catheter (inside diameter, 2 mm) was placed in the middle portion of the esophagus and connected to a pressure transducer for recording esophageal pressure. Upper airway pressure was recorded through a catheter inserted into the uppermost sidearm of the tracheal cannula. A large polyethylene tube (inside diameter, 9 mm) was inserted through the mouth and positioned, with the aid of a laryngoscope, just below the epiglottis, facing the opening of the larynx. The tube was then secured by closing the mandible and constructing a face mask around the mouth and nose with quick-setting epoxy.[20]

The RLNs were isolated between the cricoid cartilage and the fourth or fifth tracheal ring. The peripheral cut end of the nerve was then placed on a dissecting tray, covered with mineral oil, desheathed, and divided into thinner bundles by means of a pair of iridectomy scissors and watchmaker forceps, with the aid of a microscope. The activity present in each small bundle was monitored by putting the filament across a pair of platinum electrodes connected to an AC preamplifier and an oscilloscope in parallel with a loudspeaker amplifier. By further dissection, the action potentials originating from a "single" fiber were identified. The action potentials were displayed on an oscilloscope and recorded on a Gould electrostatic recorder (ES 1000) together with esophageal and upper airway pressures. In some experiments also the rate of discharge of the receptors, electronically computed, was recorded.

A similar procedure was followed to record the afferent activity of the ExtSLN. This branch of the SLN was isolated on the right side between its entrance into the larynx and the junction with the internal branch of the SLN. Subsequent procedures and experimental protocols were similar to those described for recurrent afferents.

Experimental Protocol. The experimental setup used for this protocol is illustrated in Fig 1. Single fiber activity was recorded during the following conditions.

1. Upper airway breathing (sidearms of tracheal cannula occluded, dog breathing through the oral cannula; Fig 1), in which the larynx is subjected to changes in transmural pressure, temperature and "drive."[20] We define "drive" as the effects of the spontaneous contraction of laryngeal muscles and the passive movements of the trachea (tracheal tug) due to the action of chest wall respiratory muscles.
2. Tracheostomy breathing (dog breathing through sidearm B3, all other outlets occluded; Fig 1), in which the larynx is bypassed by the airflow and therefore subjected only to the "drive."
3. Occlusion of the upper airway at end expiration in which the larynx, during inspiration, is subjected to collapsing pressure and "drive."
4. Occlusion of the trachea at end expiration, in which the larynx is subjected to the "drive."
5. Occlusion of the upper airway at peak inspiration, in which the larynx is subjected to a distending pressure.
6. Manual displacement of the larynx in both caudal and cranial directions.

Filaments that were silent during tracheostomy breathing and upper airway breathing were nevertheless challenged with the other maneuvers. By comparing the discharge patterns of a receptor during these experimental conditions, it is possible to characterize the nature of the most effective stimulus, ie, pressure, temperature, or "drive." A flow of room air (25°C, 55% relative humidity) was passed through the isolated upper airway (by inflating the cuff of a Foley catheter introduced through sidearm B2 of the tracheal cannula; Fig 1) as an additional way to ascertain the presence of cold receptors.

The location of individual receptors was established by gently probing the external laryngeal structures (cricothyroid muscles, cricothyroid membrane, etc) with a cotton applicator soaked in saline and probing the laryngeal mucosa with a partially inflated cuff of a Foley catheter (14F) inserted through sidearm B2 of the tracheal cannula (Fig 1). For two receptors the mucosal location was determined by probing the exposed laryngeal lumen with a cotton applicator.

Some of the RLN receptors, deemed to be located within the subglottic mucosa (response to light probing), were also challenged with deionized water, an isotonic solution of dextrose, and cigarette smoke.

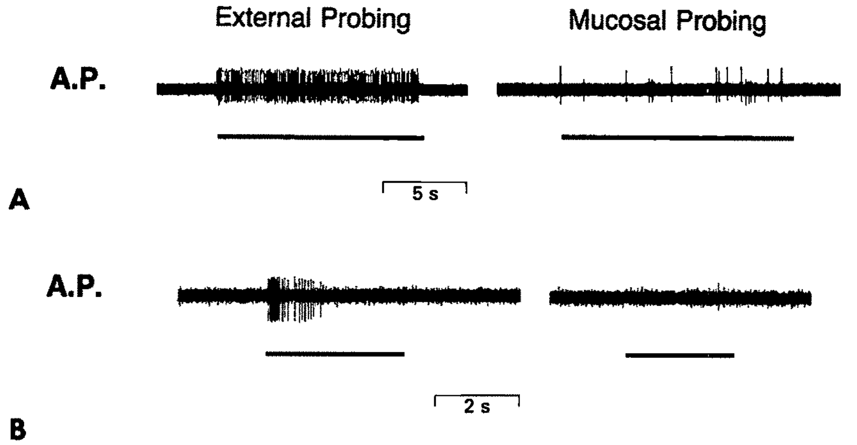

Fig 2. A) Slowly and B) rapidly adapting behavior of two receptors with fibers in external branch of superior laryngeal nerve. Probing (as indicated by thick lines) of external laryngeal structures was most effective stimulus for both endings. A.P. — action potentials.

The upper airway was isolated, and 10 mL of either deionized water or isotonic dextrose at 37°C was instilled into the laryngeal lumen with a polyethylene catheter inserted through sidearm B1. Two hundred milliliters of cigarette smoke (research cigarette series 2A1, laced with nicotine, developed by the University of Kentucky Tobacco and Health Institute) was administered into the larynx through the oral cannula while the upper airway was isolated and the dog breathed through the tracheostoma.

RESULTS

External Branch of Superior Laryngeal Nerve. Of 39 receptors studied, only 6 were active in the absence of any apparent stimulation (as during the expiratory pause). Thirty-eight receptors were stimulated by probing the external laryngeal structures, and 5 of them, although to a lesser extent, also by probing the laryngeal mucosa (Figs 2 and 3). The remaining receptor was stimulated only by mucosal probing.

The discharge characteristics of 29 endings were assessed by applying a sustained pressure on the external laryngeal structures. Twenty-one endings exhibited a slowly adapting discharge, while the remaining 8 had a short-lasting burst of activity (Fig 2, left panel).

Ten of the 39 receptors exhibited a respiratory modulation. Of these receptors, laryngeal collapsing pressure stimulated 8 (Fig 3) and inhibited 1. One receptor was recruited only by tracheal occlusion. Thirteen receptors were challenged with positive pressure: 3 were stimulated, 1 was inhibited, and the rest were unaffected. The effect of laryngeal displacement was tested in 14: half of them were activated by both caudal and cranial displacements (Fig 3). None of the receptors studied was found to be stimulated by cooling of the laryngeal lumen.

Twenty-five of the 39 receptors were localized: 19 were located within the cricothyroid muscle, 1 at the insertion of the cricothyroid muscle on the thyroid cartilage, 3 within strap muscles, and 2 between the cricoid cartilage and the first tracheal ring.

Recurrent Laryngeal Nerve. Of the 30 receptors studied, only 2 were active in the absence of any apparent stimulation (as during the expiratory pause). All responded to probing of the laryngeal mucosa: 29 were stimulated and 1 was inhibited. Sustained mechanical probing elicited a slowly adapting discharge with regular interspike intervals in 12 recep-

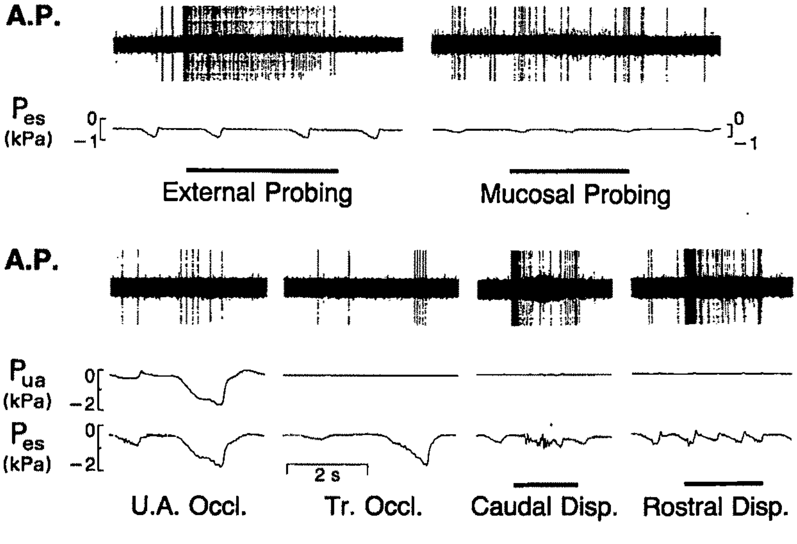

Fig 3. Behavior of receptor with fiber in external branch of superior laryngeal nerve. This ending was stimulated by both external and mucosal probing; former stimulus was more effective. Negative pressure in upper airway (upper airway occlusion [U.A.Occl.]) and longitudinal displacement of larynx (tracheal occlusion [Tr. Occl.] and caudal and rostral displacement [Disp.]) also stimulated this receptor. Longitudinal displacement of trachea introduced artifacts in esophageal pressure (Pes) tracing. A.P. — action potentials, kPa — kilopascals, Pua — upper airway pressure.

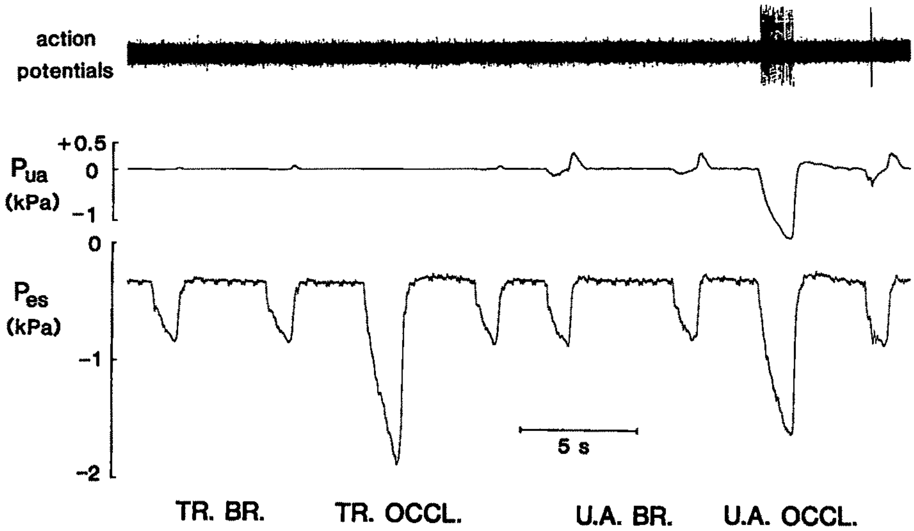

Fig 4. Receptor with fiber in recurrent laryngeal nerve stimulated by negative pressure. This receptor responds only to large changes in laryngeal collapsing pressure, as during occlusion of upper airway. Pua — upper airway pressure, kPa — kilopascals, Pes — esophageal pressure, TR. BR. — tracheostomy breathing, TR. OCCL. — tracheal occlusion, U.A. BR. — upper airway breathing, U.A. OCCL. — upper airway occlusion.

tors and a short-lasting burst of activity with irregular interspike intervals in the remaining 18. Probing of the external laryngeal structures activated 6 receptors and inhibited 1.

Among the 30 receptors studied, 7 showed a respiratory modulation. During an inspiratory effort performed at end-expiratory volume against an occluded upper airway, 5 of these receptors increased and 2 decreased their discharge, ie, negative pressure stimulated 5 (Fig 4) and inhibited 2. Positive pressure stimulated 2 of these endings (Fig 5).

Laryngeal displacement affected only a few of the 14 receptors tested: cranial displacement inhibited 2, whereas caudal displacement stimulated 2 and inhibited 1 (Fig 5).

Six slowly adapting and 10 rapidly adapting receptors were challenged with water: 4 of the former and 2 of the latter type were stimulated (Fig 6). When the water-responsive receptors were tested with an isotonic solution of dextrose only, 1 slowly adapting receptor was stimulated. Five of the slowly adapting and 8 of the rapidly adapting receptors that were challenged with water were also challenged with smoke: 1 of each type was stimulated. None of the receptors was stimulated by laryngeal cooling.

The location was ascertained for 20 endings: 17 were located below and 1 above the glottis, 1 within the cricothyroid muscle, and 1 in the cricothyroid membrane.

In the course of this study, in addition to the laryngeal endings, we encountered four receptors that were located in the extrathoracic trachea.

DISCUSSION

An observation that became readily apparent during recording from either the RLN or the ExtSLN was the scant level of overall afferent activity as compared to the abundant activity present in the IntSLN.[21] This is consistent with the relatively scarce number of units responsive to pressure, tracheal tug, and irritant stimuli, and to a complete lack of the specific cold endings. The absence of any noticeable respiratory modulation in the recordings from the whole nerves, even when the dog breathed through the upper airway or performed efforts against an occluded upper airway, is a further illustration of the paucity of afferent endings.

Since the afferent activity of the RLN and the ExtSLN was recorded from their peripheral cut ends, the intrinsic laryngeal muscles ipsilateral to the recording side were denervated. Therefore, while stimuli such as pressure, temperature, tracheal tug, and local probing were preserved, any influence de-

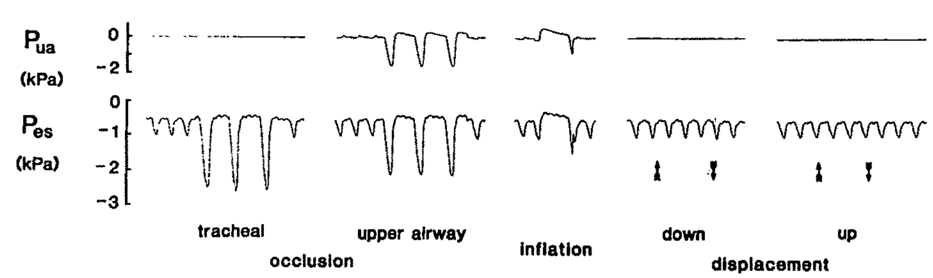

Fig 5. Receptor with fiber in recurrent laryngeal nerve having tonic activity and respiratory modulation. Traces are, top to bottom: time in seconds, instantaneous rate of discharge (IMPS/S), upper airway pressure (Pua) in kilopascals (kPa), and esophageal pressure (Pes). This receptor was inhibited by negative pressure and stimulated by positive pressure. Caudal displacement of larynx stimulated it and cranial displacement inhibited it.

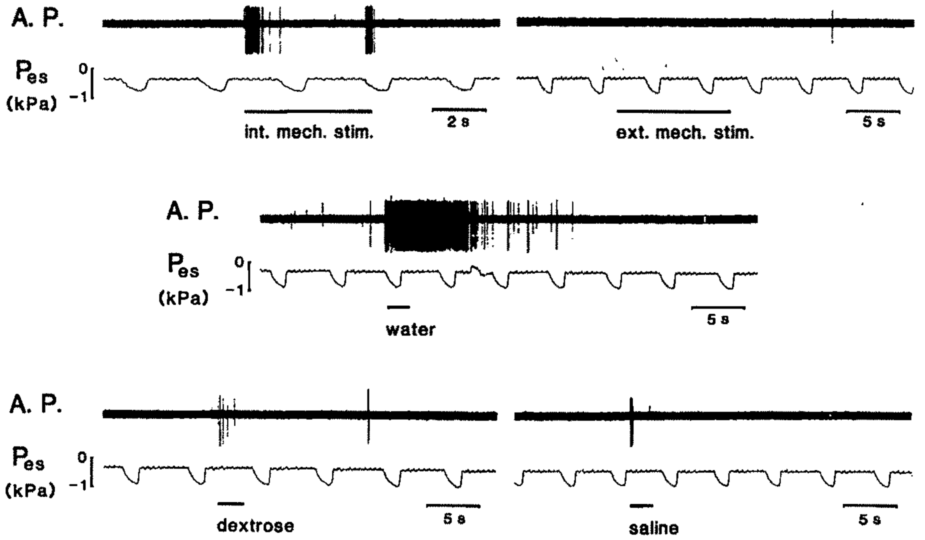

Fig 6. Recurrent laryngeal nerve receptor responding with rapid-adapting discharge at "on" and "off" of intraluminal cuff inflation. Distortion of larynx exerted from outside does not activate this ending. Intraluminal instillation of deionized water (10 mL) stimulates receptor with short delay and short-duration discharge. Equal amount of saline and isotonic dextrose causes only short burst of action potentials (A.P.) owing to mechanical stimulation. Pes — esophageal pressure, kPa — kilopascals.

riving from laryngeal muscle contraction was lost. However, recording from one nerve (eg, RLN) while leaving the other intact (eg, ExtSLN) made it possible to determine that ipsilateral partial denervation did not appreciably affect the activity being recorded. This observation indicates that some of the laryngeal mechanoreceptors, perhaps owing to a well-circumscribed location, have a well-defined mechanical transducing property. A similar selectivity has been observed for some of the mechanoreceptors recorded from the IntSLN.[22]

The specific cold receptors present in the nasal cavity[23,24] and on the vocal folds[8] are not present in the regions supplied by the RLN and ExtSLN. This finding is in line with the absence of similar endings in the extrathoracic trachea and elsewhere in the tracheobronchial tree. Moreover, the inhibitory effect of cooling on various types of mechanoreceptors diminishes from the larynx to the trachea.[9,25] It seems, therefore, that the neural information related to cooling is mostly concentrated in the more proximal regions of the respiratory tract.

Pressure-responsive receptors (Figs 4 and 5) were found in a similar proportion among RLN and ExtSLN afferents. In each case, receptors activated by negative pressure outnumbered those stimulated by distending pressure, similar to the findings in the IntSLN.[20] However, pressure receptors with fibers either in the RLN or the ExtSLN were definitely scarce.

There appears to be a considerable difference in the location of RLN and ExtSLN receptors. The ExtSLN endings were found predominantly in association with the external laryngeal structures. Most RLN afferents, on the other hand, were found in the subglottic region in a location deemed to be superficial in light of the gentle probing that activated them (Fig 7). This supports the previous observations of Suzuki and Kirchner[26] based on topical anesthesia of the feline larynx.

Several of the RLN endings, for their short-lived and irregular burst of action potentials in response to probing, have the characteristics of rapidly adapting mechanoreceptors. Two of 10 responded with a short-delay, short-duration discharge when challenged with deionized water (Fig 6). These endings were not activated by an isotonic solution of dextrose, responding, therefore, to a decrease in osmolality rather than to a lack of chloride ions.[27] Such behavior is distinctly different from that reported for similar receptors of the IntSLN,[27] but similar to that described for bronchial rapidly adapting receptors.[28] Four RLN endings that had a "regular," slowly adapting discharge in response to light probing responded to water with a long-lasting discharge. That three of them were not stimulated by isotonic dextrose indicates that their response to water is due to hyposmolarity. That the remaining one was activated also by isotonic dextrose suggests

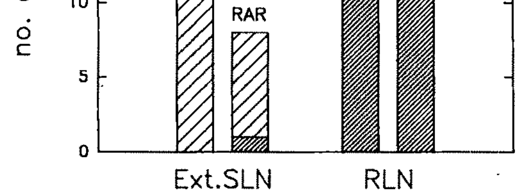

Fig 7. Slowly (SAR) and rapidly (RAR) adapting receptors found in external branch of superior laryngeal nerve (Ext. SLN) and recurrent laryngeal nerve (RLN). Note that Ext. SLN endings are mostly slowly adapting and preferentially or uniquely stimulated from outside of larynx, whereas RLN endings are more commonly rapidly adapting and preferentially or uniquely stimulated from luminal side.

a responsiveness to a lack of chloride ions.[27] Whereas the former behavior is typical of this type of "regularly" firing receptor with fibers in the IntSLN, the latter is not.

The endings showing a rapidly adapting irregular discharge in response to probing have the characteristics of receptors responding to irritant stimuli. However, when tried with cigarette smoke only one of eight showed a clear activation. Also, one of the other type of receptors, with a "regular" and slowly adapting discharge, was stimulated by smoke. Therefore, it seems that receptors that apparently have some properties of endings mediating defensive responses are atypical in other respects. To what extent these atypical characteristics modify defensive reflexes elicitable from this area is not clear.

The results of this study allow only an indirect assessment of the presence in the RLN and the ExtSLN of fibers from myotatic mechanoreceptors of the intrinsic laryngeal muscles. Most of the unit activity we recorded from the ExtSLN appeared to originate within the cricothyroid muscle and showed a slowly adapting discharge in response to a sustained compression exerted on this muscle. A few other endings appeared to be associated with the strap muscles, and only one could be activated by probing the subglottic mucosa. Whether the endings associated with the cricothyroid muscle are myotatic mechanoreceptors is difficult to decide, but the fact that they were consistently activated by localized compressions and dislocations of this muscle makes this a very likely possibility. Altogether, the present results indicate that most of the afferent activity recorded from the ExtSLN originates from the cricothyroid muscle, and only one receptor is identifiable as a mucosal ending. These findings are at variance from previous reports that found, in the cat, cricothyroid muscle afferents running in the RLN and not in the ExtSLN.[14,29,30] Our present results on RLN afferents actually revealed the presence of only one ending associated with the cricothyroid muscle. Also, the paucity of mucosal endings found in the ExtSLN discloses another difference between canine and feline larynges; in fact, in the cat the entire mucosa overlying the cricothyroid membrane is innervated by the ExtSLN.[30] However, these findings are not supported by a recent histochemical study in the cat by Tanaka et al[31] or a previous electrophysiologic study by Sumi[32] in the same species.

Probing of the subglottic mucosa appeared to be the most effective stimulus for eliciting a discharge in RLN afferents, and the majority of them responded with a short burst of irregularly spaced action potentials, ie, a response characteristic of rapidly adapting receptors. Some of the endings with a regular discharging pattern could be muscle proprioceptors, but no definite supporting evidence can be obtained from our results. We should also be aware that in the dog some afferent activity recorded from the IntSLN could very well be interpreted, from a functional point of view, as deriving from myotatic mechanoreceptors of intrinsic laryngeal muscles whose motor supply is provided by the RLN and the ExtSLN.[22]

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IMAGING CASE STUDY OF THE MONTH

PULSATILE TINNITUS FROM A DURAL ARTERIOVENOUS FISTULA

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CASE REPORT

A 50-year-old woman presented with headaches and left pulsatile tinnitus that she first noticed several months before. The tinnitus had been increasing in intensity, was now constant, and often caused the patient to awaken from sleep. There was no antecedent history of trauma. No visual changes were associated with the headaches.

Examination disclosed no apparent cutaneous abnormalities. No neurologic deficits were present. A bruit could be heard that was loudest over the left occiput. Laboratory data were normal.

Radiographic evaluation included computed tomography and magnetic resonance imaging of the head; results were normal. Angiography demonstrated a vascular malformation in the region of the left transverse sinus. Numerous meningeal arteries from both the internal and external carotid arteries were feeding the malformation. Rapid shunting of blood to the venous side was apparent in the left transverse sinus. The nidus of the malformation appeared to lie in the walls of the sinus. The findings were characteristic of a dural arteriovenous fistula.

The major arterial supply was from transmastoid branches of the left occipital artery and from branches of the left middle meningeal artery (see Figure, A-D). Highly selective catheterizations with embolization using polyvinyl alcohol foam particles (Pacific Medical Industries, San Diego, Calif) were performed in the two major feeders. This procedure resulted in immediate relief of symptoms despite the persistence of several small feeders. Two years after the procedure, the patient remains asymptomatic.

DISCUSSION

Dural arteriovenous fistulas (DAVFs) are vascular malformations characterized by arterial-venous shunting from meningeal arteries into one of the intracranial venous sinuses. Typically, multiple small meningeal arterioles appear to converge on the walls of a dural sinus, shunting blood into the sinus. The venous filling is very rapid as in a true fistula or arteriovenous malformation. Symptoms are usually headaches and tinnitus. Less commonly, papilledema and visual deficits, various cranial nerve dysfunctions, and occasionally subarachnoid hemorrhage may result, although these more serious complications are unlikely except in cases in which cortical venous drainage or venous outflow obstruction becomes a feature.[1-6] Probably DAVFs are acquired lesions, not true congenital malformations, resulting from a sinus thrombosis that recanalizes.[7,8] In the patient with pulsatile tinnitus, DAVFs must be distinguished from paraganglioma, an aberrant course of the internal carotid artery, and a dehiscent (high) jugular bulb.

Usually the goal of therapy is a symptomatic cure. Most DAVFs do not require complete cure.[4] Total obliteration with embolization or total surgical resection can be reserved for those high-risk cases in which cortical venous drainage and/or venous outflow obstruction are present.

Embolization can be performed via a venous, arterial, or combined approach.[9-11] If the venous approach is taken the sinus must be totally obliterated along the entire length of involvement to prevent undesirable venous collateral formation. Occlusion of the involved sinus can be curative. Arterial embolization is also frequently satisfactory, and partial embolization of the arterial supply, as in this case, may be sufficient. The major risk of embolization is the potential for occlusion of normal vessels that serve important territories. For example, in the case presented here, embolization included the petrosal branch of the middle meningeal artery, which can contribute to the facial nerve. Test injections with lidocaine prior to embolization did not provoke a palsy, and neither did the subsequent embolization.[12] Also, the occipital artery angiogram initially demonstrated a well-developed C1 segmental artery that anastomosed with the vertebral-basilar system (see Figure, E). To avoid vertebral-basilar emboli the microcatheter was positioned distal to the dangerous anastomosis for the deposition of emboli.[13] Again, no neurologic deficits were incurred.

In summary, DAVFs are uncommon lesions that typically present with pulsatile tinnitus. They must

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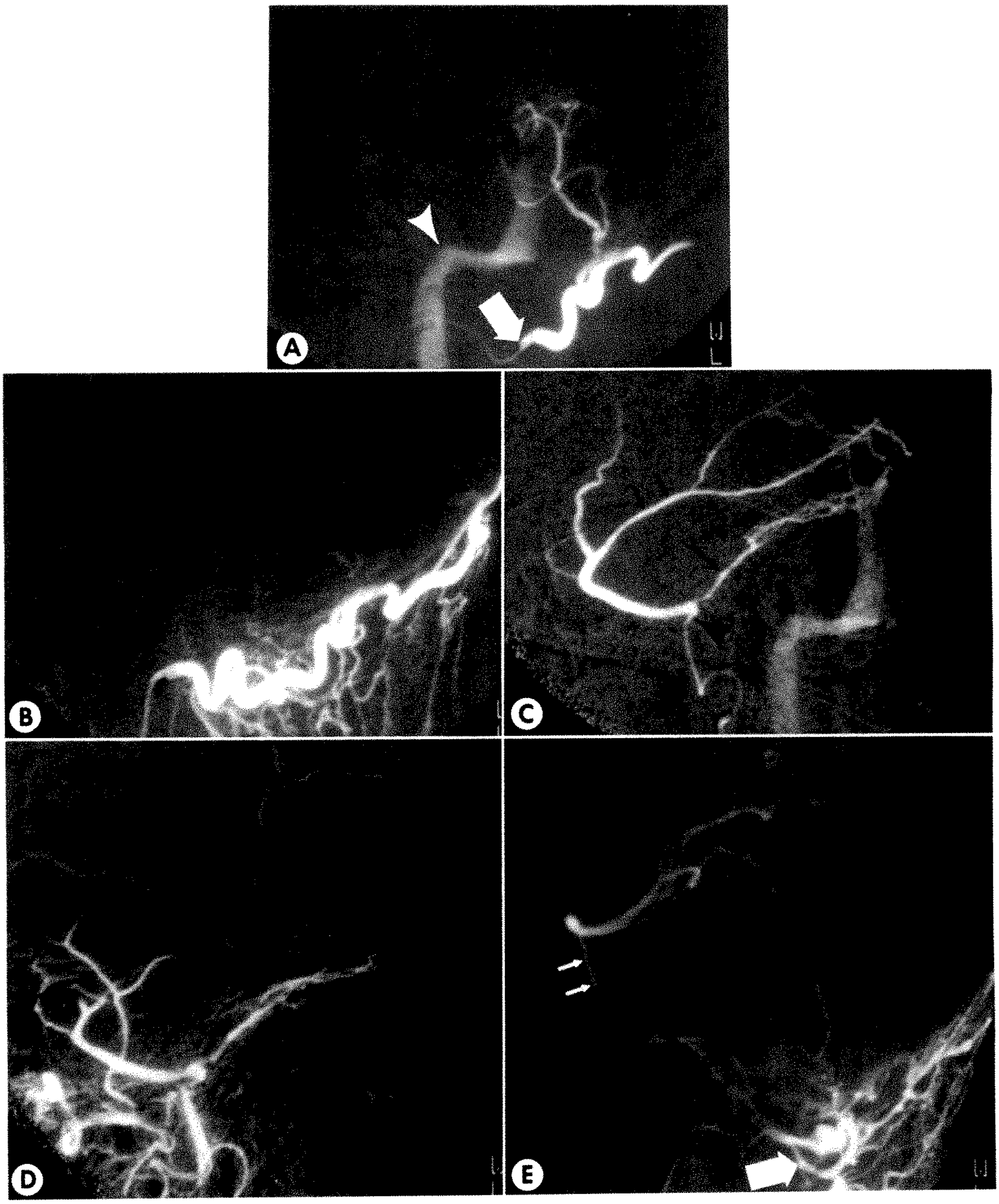

Dural arteriovenous fistula (DAVF) of left transverse sinus. Lateral angiographic views. A) Occipital artery angiogram. Large transmastoid branch arborizes into many small feeders that shunt blood into sigmoid sinus at junction with transverse sinus. Jugular vein is well opacified. Arrow — tip of microcatheter in occipital artery, arrowhead — jugular bulb. B) Occipital artery angiogram taken after embolization. DAVF no longer fills, and occipital artery is preserved. C) Superselective middle meningeal artery angiogram. Tip of microcatheter (arrowhead) is at foramen spinosum. Both petrosal branch (single arrow) and posterior division (double arrows) of middle meningeal artery feed DAVF. Rapid venous sinus opacification is present. D) Middle meningeal artery angiogram taken after embolization. Tip of microcatheter is in same position as in C, but contrast has refluxed into internal maxillary artery. Middle meningeal artery branches no longer supply DAVF. E) Occipital artery angiogram. Tip of microcatheter (single arrow) is positioned such that preferential flow is into C1 segmental artery and vertebral-basilar system (double arrows) rather than into DAVF. This is dangerous position from which to embolize. For embolization, microcatheter was placed more distally (see A), beyond dangerous anastomosis.

be distinguished from other causes of tinnitus. The clinical course is usually benign. In cases requiring treatment, endovascular therapy (embolization) is frequently beneficial.

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CONFERENCE ON ASSISTIVE LISTENING DEVICES
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A Conference on Assistive Listening Devices, Tutorials, Applications, and Research will be held June 12-14, 1992, at The University of Iowa, Iowa City, Iowa. For information on registration and accommodation, contact the Conference Center, University of Iowa, Memorial Union, Iowa City, IA 52242; (319) 335-3231, Fax (319) 335-3407. For information on Call for Papers, contact Regina Tisor (319) 356-2471, Fax (319) 356-4547. We have applied for Continuing Education Units from ASHA and HAIC.

Ann Otol Rhinol Laryngol 100:1991

PATHOLOGY CONSULTATION

SALIVARY NEOPLASMS OF THE JAW BONES WITH PARTICULAR REFERENCE TO CENTRAL MUCOEPIDERMOID CARCINOMAS

JANET M. BRUNER, MD JOHN G. BATSAKIS, MD
HOUSTON, TEXAS

Intragnathic salivary-type neoplasms are few in number, are of debated histogenesis, and are most often classified as mucoepidermoid carcinomas. Two generating tissues have been proposed for the origin of these central (intraosseous) tumors: enclaves of salivary tissue and a metaplasia of the linings of odontogenic cysts. They are not mutually exclusive. Although most mucoepidermoid carcinomas have been low grade, these neoplasms nonetheless have a very respectable recurrence rate and can be fatal.

Primary nonodontogenic epithelial neoplasms of the gnathic bones are rare lesions and should always be approached with considerable diagnostic circumspection. When metastases to the jaw bones and direct extension from mucosal or cutaneous primaries are excluded, there remain central (intraosseous) tumors of presumed salivary origin. Mucoepidermoid carcinomas are the class of tumor most often reported. Much fewer in number are salivary adenomas (pleomorphic and monomorphic adenomas) and adenoid cystic carcinomas.

Presumed origin from a maternal salivary gland tissue is predicated on the presence of the tissue within the jaw bones. Evidence in support for centrally placed salivary tissue is strongest for the mandible. Salivary gland inclusions mostly occur in bone spaces located near the angle of the mandible, where they have a rather consistent position and radiographic appearance.[1] Only a few inclusions have been found in the anterior mandible (in the incisor region or between the canines and first molars).[1]

No single theory of histogenesis is applicable to all cases.[2,3] True examples of central pleomorphic or monomorphic adenoma and adenoid cystic carcinoma do not arise from a metaplasia of odontogenic cysts and must therefore take origin 1) from enclaved retromolar mucous glands during embryologic development of the jaw bones, 2) from submandibular and sublingual glands closely apposed in bony defects or cavities in the lingual cortex of the mandible, or 3) from parts of these latter glands that have been embryologically evaginated.

CENTRAL (INTRAOSSEOUS) MUCOEPIDERMOID CARCINOMA (N = 66)

Gnathic bone involved	48 in mandible, 18 in maxilla
Age of patients	1 to 85 y (mean, 51 y)
Sex of patients	36 female, 28 male, 2 not indicated in report
Radiographic findings	Varied and nondiagnostic; unilocular or multilocular radiolucencies
Recurrences	17/66 (25.8%)
Metastases	8/66 (12.1%)
Death due to neoplasm	6/66 (9.1%)

From data presented by Waldron and Koh.[4]

A third speculative source has also been proposed for the beginnings of central mucoepidermoid carcinomas: metaplasia in an odontogenic cyst or epithelial rests of Malassez.[3,4]

The commonly accepted criteria for the diagnosis of a central mucoepidermoid carcinoma are also applicable to other histologic types of salivary gland tumors of the jaw bones. They include 1) intact cortical plates, 2) radiographic evidence of bone destruction, 3) exclusion of another primary that in its metastasis could histologically mimic the central tumor, 4) exclusion of an odontogenic tumor, and 5) histopathologic confirmation. A detectable intracellular mucin is often added as confirming evidence of a mucoepidermoid carcinoma. In the appropriate context, this finding is helpful, but it is

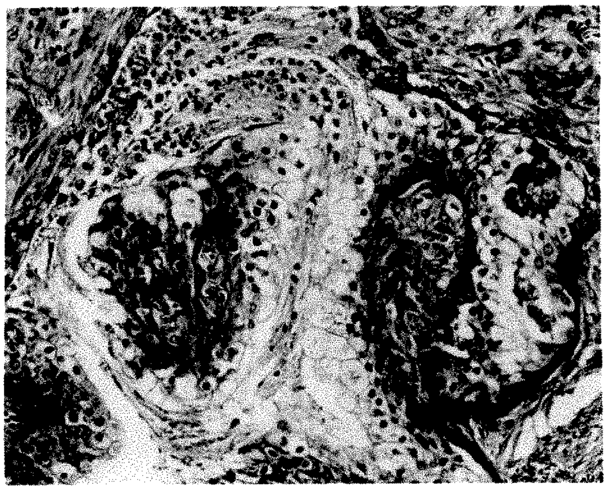

Fig 1. Low-grade mucoepidermoid carcinoma of mandible with histologic appearance like that seen in mucoepidermoid carcinomas of major and minor salivary glands (H & E, original x140).

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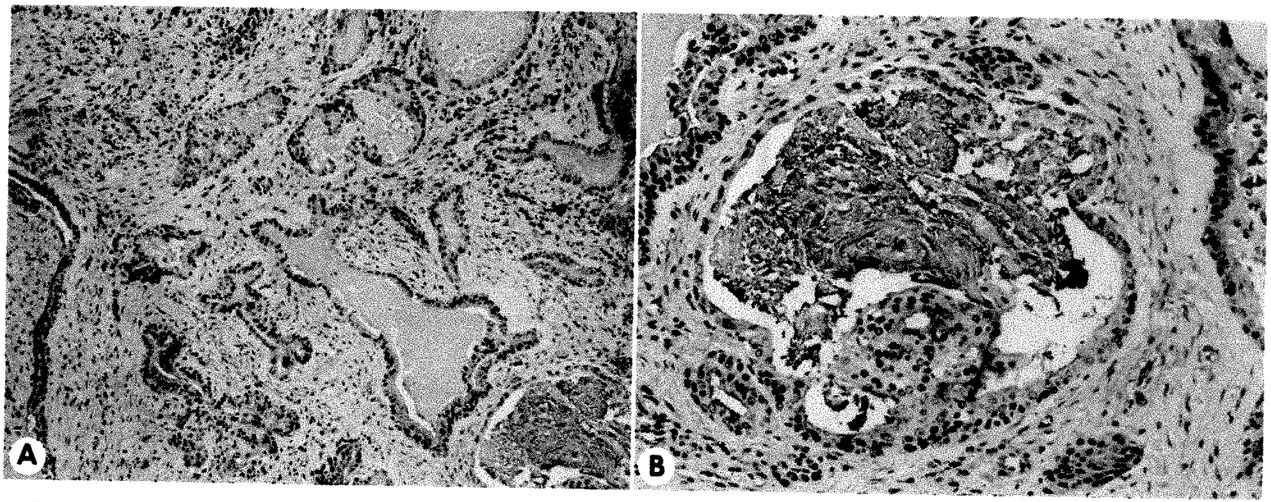

Fig 2. Intraosseous, low-grade mucoepidermoid carcinoma of mandible (H & E). It is this appearance that suggests origin from odontogenic cyst rather than from salivary tissue and raises differential diagnosis of sialodontogenic cyst. A) Original x40. B) Original x200.

not diagnostic. Mucinous metaplasia is known to occur in odontogenic cysts, and intracellular mucin is not restricted to only mucoepidermoid neoplasms. Intact cortical plates of the involved bone are certainly strong evidence for an intraosseous origin of a salivary-type neoplasm, but may not be a completely reasonable requirement, especially for tumors of the thinner maxilla as opposed to the mandible. Waldron and Koh[4] accept cortical plate defects in a mandible overlying the radiolucent lesion if it is unassociated with an obvious soft tissue tumor and if the periosteum is intact.

Only central mucoepidermoid carcinomas have sufficient reported numbers to allow clinicopathologic analysis.[4] Other salivary neoplasms are presented in single case reports or small series with insufficient documentation.[5,6] Waldron and Koh[4] indicate that at least 66 cases of central mucoepidermoid carcinoma of the jaws have been reported (see Table[4]), with the mandible involved almost three times as often as the maxilla. The great majority of the carcinomas have been low grade (Fig 1) and preponderantly cystic.[4]

Treatment has ranged from enucleation or curettage to en bloc or radical excision. Several patients have also received radiotherapy.[4] Enucleation or curettage, with or without electrocoagulation, is associated with the highest recurrence rate (45%), but no method of treatment is completely free of that event. As recorded in the review by Waldron and Koh, metastases and death were highest in patients undergoing hemimaxillectomy or hemimandibulectomy.[4] This finding likely reflects an advanced stage of disease at the time of initial treatment.

The principal differential diagnoses for gnathic mucoepidermoid carcinomas are metastases from other primary carcinomas and cystic odontogenic lesions, particularly the sialodontogenic cyst (glandular odontogenic cyst, mucoepidermoid cyst).[4,7,8] These lesions enter the differential diagnosis in instances in which the carcinoma manifests sufficient histologic differences from the prototypes of extraosseous mucoepidermoid carcinomas (Fig 2).

The sialodontogenic cyst is relatively unknown to pathologists, and its features overlap with those of some low-grade mucoepidermoid carcinomas.[4,7,8] For that reason, Waldron and Koh[4] wonder if the sialodontogenic cyst is not just part of a spectrum of central mucoepidermoid carcinoma.

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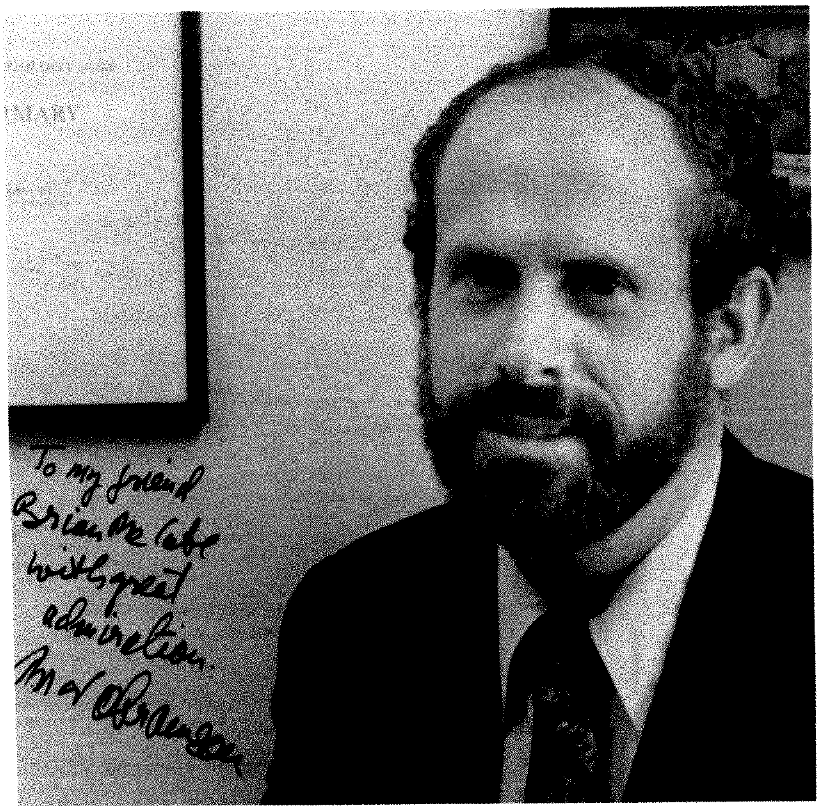
OBITUARY
MAXWELL ABRAMSON, 1935-1991



Maxwell Abramson is gone, the victim of a tragic fluke accident in Hawaii, and we, the legion of his friends and admirers, are all lesser for it.

This photograph of Max is printed here not for the inscription (except for how it represents his generosity of spirit) but for how it represents him as I will always remember him in our frequent deep discussions of scientific and educational issues in otolaryngology — pensive, imaginative, and resourceful.

I first saw Max when he made his epic presentation at an Academy committee program, which group was the predecessor to the present Association for Research in Otolaryngology. He made the most significant contribution to our knowledge of the bone-destroying mechanism of cholesteatoma in this century. When he made this presentation my colleagues and I at Iowa decided to make him an offer he couldn't refuse, to join our department. He did this work under distinguished mentors at Harvard, Dr Jerry Gross and Dr Harold Schuknecht.

He remained with us at Iowa for 8 years, rising from assistant professor to full professor in just 6 years, which is also remarkable. He made many more significant contributions. He then gained his ultimate goal, a department chairmanship. I very much regretted his loss, but again resigned myself that this seems to be my lot in academic life, and gave him my full support and congratulations.

Maxwell's death is really not the end of him; we should all seek to continue him. Max knew well, as we all in medicine know, death is a part of life. As Shakespeare wrote,

Of all the wonders I have yet heard,
  It seems to me most strange that men should fear
Seeing that death, a necessary end
  Will come when it will come.

So though we sorrow, we must more than that joy in, and celebrate, the life of Maxwell. On the personal side, he had a remarkable life. He married a beautiful and talented woman, Marcia, who went on to professional life and achievement in her own right. They raised three children, who at last report may exceed their parents in professional productivity.

Then there was Max himself, whose fresh charm put an amiable, human face on the often arduous affairs of everyday and academic life.

Brian F. McCabe, MD

LETTERS TO THE EDITOR

To the Editor:

I read with interest the article by Dr Bartels (Facial Nerve and Medially Invasive Petrous Bone Cholesteatomas, *Ann Otol Rhinol Laryngol* 1991;100:308-16) reporting his experience with eight cases of extensive temporal bone cholesteatomas that invaded the labyrinth and fallopian canal. The literature review, case reports, imaging studies, and recommendations were presented well, making this a valuable addition to the literature.

I found it interesting that muscle and soft tissue flaps were used to obliterate the mastoid and middle ear defects when necessary to prevent cerebrospinal fluid leaks postoperatively. Although radical obliteration of the eustachian tube, middle ear, mastoid, and ear canal with oversewing of the auditory meatus is certainly appropriate in these selected cases, abdominal fat seems much more useful as obliterating tissue. The use of fat is commonly done to obliterate defects following translabyrinthine removal of acoustic neuromas and other cerebellopontine angle lesions because of the ease of harvesting ample amounts of tissue with minimal operating time and morbidity. More important for these cases, however, is the superior tissue contrast between fat and cholesteatoma detected by magnetic resonance imaging (MRI). Fat carries high signal intensity on T1-weighted images that lessens considerably on T2-weighted studies. This is exactly opposite to cholesteatomas, which are of medium intensity on T1 and brighten on T2. Scar tissue, muscle, and soft tissue can be variable on MRI and are more similar to cholesteatoma than fat. Since imaging is the only way to follow patients with radically obliterated temporal bone defects, and MRI is the test of choice in this situation, recurrence of cholesteatoma is more easily detected if fat is used as the obliterating material.

Recently, I've had personal experience with an ear radically obliterated with abdominal fat following extensive cholesteatoma removal. Follow-up MRI 12 months later clearly shows the mastoid, middle ear, and auditory canal completely filled with fat and no sign of cholesteatoma. Although this may seem a minor point, since follow-up by MRI is critical in these patients, appropriate tissue should be used to maximize one's chance of detecting recurrence at later studies.

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To the Editor:

I appreciated the comments of Dr Hodgson. He suggests that free abdominal fat grafts are superior to local muscle rotation flaps in obliterating a temporal bone cavity because fat images more distinctly different from cholesteatoma than muscle does with magnetic resonance imaging.

Fat to obliterate a temporal bone defect after skull base surgery has wide acceptance in management of clean, limited-size wounds. In extensive skull base surgery, infected abdominal fat grafts can be a problem. The application of vascularized local flaps and microvascular free flaps to reconstitute extensive skull base defects has lessened the complication rate (infection and cerebrospinal fluid leak) for skull base surgery. Thus, methods other than free fat grafts are preferred in larger skull base defects.[1,2] I have had the opportunity to care for two patients (one from another community) with infected abdominal fat grafts to the temporal bone. The difficulties of managing a wound with fibrosis and a fragile facial nerve do not need description.

Cholesteatoma ears, typically, are contaminated wounds. Because of the potential for bacterial colonization of free fat grafts to such wounds, this surgeon virtually always elects local soft tissue rotation flaps for total temporal bone obliteration. As well, when extensive neoplastic disease invades the temporal bone and surrounding soft tissue, we elect to use microvascular free flaps with muscle, fat, and skin to obliterate the defect and reconstitute skin coverage. We assiduously avoid large nonvascularized tissue grafts in wounds not clearly considered clean. We have had no perioperative infections in this group of patients.

The potential for residual disease after cholesteatoma removal is a not insignificant concern that has plagued temporal bone obliteration surgery for decades.[3] Rambo's[4,5] success with temporal bone obliteration was followed by a report by Fritz and Crawford[6] of delayed appearance of extensive residual disease. In spite of the potential for residual disease, others have been willing to obliterate severely diseased temporal bones, often with muscle flaps. Gacek[7,8] emphasized the necessity for thorough temporal bone disease removal prior to obliteration. He described local soft tissue flaps for obliteration, allowing a fat graft when necessary.

In summary, while free fat grafts are a reasonable technique to apply to clean, limited temporal bone defects, this author is inclined to use vascularized tissue rotation flaps or vascularized free grafts for larger wounds that are potentially contaminated. Since cholesteatoma is a benign, slow-growing disease, the patient can tolerate residual disease growth over a sufficient period of time for repeat magnetic resonance imaging to confirm a growth pattern consistent with cholesteatoma.

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BOOK REVIEWS

Tympanoplasty. Osteoplastic Epitympanotomy

Horst L. Wullstein and Sabina R. Wullstein. Hard cover, illustrated, indexed, 205 pages, 1990. Georg Thieme Verlag/Thieme Medical Publishers, New York, NY, $129.

The crux of this volume undoubtedly emanated from Horst Wullstein's study of osteitis of the inner ear capsule in preparation for his 1948 publication, *Die Klinik der Labyrinthitis und Paralabyrinthitis auf Grund des Rontgenbefundes* (George Thieme Verlag, Stuttgart, Germany). He began those investigations, utilizing radiology studies, in the late 1930s and continued through the pivotal time when antibiotic therapy altered the natural course of inflammatory processes. The broad and detailed knowledge of the anatomy and pathoanatomy of the temporal bone he gained became the foundation of the one-stage tympanoplasty in the early 1950s. These concepts are elucidated in this volume, the 1990 English edition of the original 1986 German publication.

The authors described effective aeration of the middle ear, around the promontory, to supply the round window membrane and the epitympanum, and the creation of central windows to assess the state of pathologic changes in the temporal bone and to correct conditions arising from aeration problems. The latter category includes deepening of the hypotympanum, flattening of the round window niche, and Sabina Wullstein's 1970 introduction of the osteoplastic epitympanotomy. A word of caution is required for those who might consider performing these operations. In preparing effective aeration pathways (deepening of the hypotympanum) in the middle ear, a constant awareness of the proximity of the jugular bulb must be maintained, and the same consideration must be given the cochlea when flattening the round window niche with a drill bur.

Perhaps even more valuable than the surgical procedures described in this text is the wealth of knowledge it conveys regarding temporal bone anatomy and pathology. One example of the extraordinary grasp of these elements Horst Wullstein acquired in preparation for his 1948 radiology studies and his experiences since is the exceptional explanation of the cholesteatoma cell tract system extending throughout the entire temporal bone. Other examples include discussions of the functional effects of subacute otitis (with middle ear fibrosis), tympanosclerosis, cholesterol granuloma, and retraction atelectasis. It is most likely that the understanding of the histopathology involved in the conditions mentioned was provided by the insight of Sabina Wullstein.

The inclusion of "osteoplastic epitympanotomy" in the title of this book may cause some prospective readers to hesitate buying it. They will do themselves a great disservice if they do not avail themselves of the abundant knowledge of temporal bone anatomy and pathology it provides. It is particularly worthwhile to see the description of the minute anatomy of the middle ear and temporal bone presented in an applicable manner, such as Koerner's septum and its significance in middle ear pathology; the function of the subiculum when providing an airstream through an inferior aeration pathway to the round window membrane; and consideration of the relationship between the position of the two window niches in estimating the depth of the sinus tympani. Sterling microphotographs, illustrations, and diagrams are generously used by the authors to enhance such information.

Although the osteoplastic epitympanotomy is not widely used today (at least in the United States) in a number of otological procedures, such as treatment of cholesteatomas, chronic infections, and temporal bone trauma, its basic principles remain germane. This book would be an extremely worthwhile addition to the library of anyone involved in practicing or teaching otology or otolaryngology. However, the reader should not expect to learn of prostheses and their uses, as these are not discussed in this volume.

DENNIS G. PAPPAS, MD
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Surgical Anatomy of the Nose

M. Eugene Tardy, Jr, and Robert J. Brown. Hard cover, illustrated, indexed, 106 pages, 1990. Raven Press, New York, NY, $95.

Surgical Anatomy of the Nose emphasizes a conservative surgical approach to aesthetic nasal surgery. In addition, the text describes specific features of nasal anatomy that directly influence the surgical procedure, with a goal of conservation and preservation of the cartilaginous and bony skeleton.

The book begins with a presentation of the topographic anatomy and landmarks of the nose that are important in a consideration of aesthetic and reconstructive surgery of the nose. This section contains numerous diagrams and illustrations depicting important and relevant features of the nasal skeleton. The next three sections are dedicated to a discussion of the skin and subcutaneous tissues, the bony skeleton, and the cartilaginous framework. This portion of the text provides an in-depth and well-illustrated step-by-step analysis of surgical manipulations that may be entertained to accomplish specific results while preserving tissue and maintaining structural support.

Surgical Anatomy of the Nose provides a detailed text and surgical atlas demonstrating the recognized change toward conservatism that has been occurring within the field of aesthetic and reconstructive nasal surgery. This text will appeal to all surgeons performing nasal surgery. It will certainly be invaluable to residents and to practitioners during their formative years while they are developing an approach to conservative and functional nasal surgery.

JOSEPH B. JACOBS, MD
New York, New York

The Peripheral Nerve: Structure, Function and Reconstruction

Julia K. Terzis and Kevin L. Smith. Hard cover, illustrated, indexed, 176 pages, 1990. Raven Press, New York, NY.

Through the efforts of many prominent surgeons, particularly in the last 20 years, facial nerve injury and repair has maintained a high profile in recent otolaryngology literature. This text is certainly not intended primarily for otolaryngologists, whose main interest involves cranial nerve injuries. Despite this, the book contains many useful principles and techniques applicable to cranial as well as peripheral nerve injuries.

Controversies in peripheral nerve injury treatment often parallel those of cranial nerve injuries. The Sunderland and Sedden injury classifications, for example, apply equally to cranial and peripheral nerve injuries. Mechanisms of injury may be similar as well, eg, injection, irradiation and laceration, and acute and chronic compression injury. The text devotes a major portion of the second of seven chapters to these various injury types in a quite concise but heavily referenced fashion. The fifth of the seven chapters deals with actual facial nerve repair and is again very concise (10 pages). Despite its brevity, this section is well referenced, precise, and quite useful, particularly to residents in surgical specialties dealing with nerve repair and nerve injury.

This succintness, in fact, characterizes most of the text. Its thin size and terse commentary belie a career interest in the field by the authors. The 835 references enumerated at the end together with the principles espoused herein by true experts serve to recommend this book to specialists with an interest in nerve injury and repair of any kind.

JEFFREY B. BANYAS, MD
New York, New York

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MANUSCRIPT. Limit papers to a size that will make up to no more than six printed pages, figuring three doublespaced typewritten pages of text to one typeset page; see journal for estimating space required for references, illustrations, and tables. If a manuscript of slightly greater length is approved by the Editor, all typeset pages in excess of six will be charged to the author at the publisher's cost of $150 per page. Submit an original and two copies of the manuscript, typed on white bond paper with margins of at least 25 mm (1 in), double-spaced throughout, including abstract, references, tables, and legends. Begin each component on a new page in the following sequence: title page, abstract, text, acknowledgments, references, tables, and legends. Number pages consecutively in the upper right corner, beginning with the title page.

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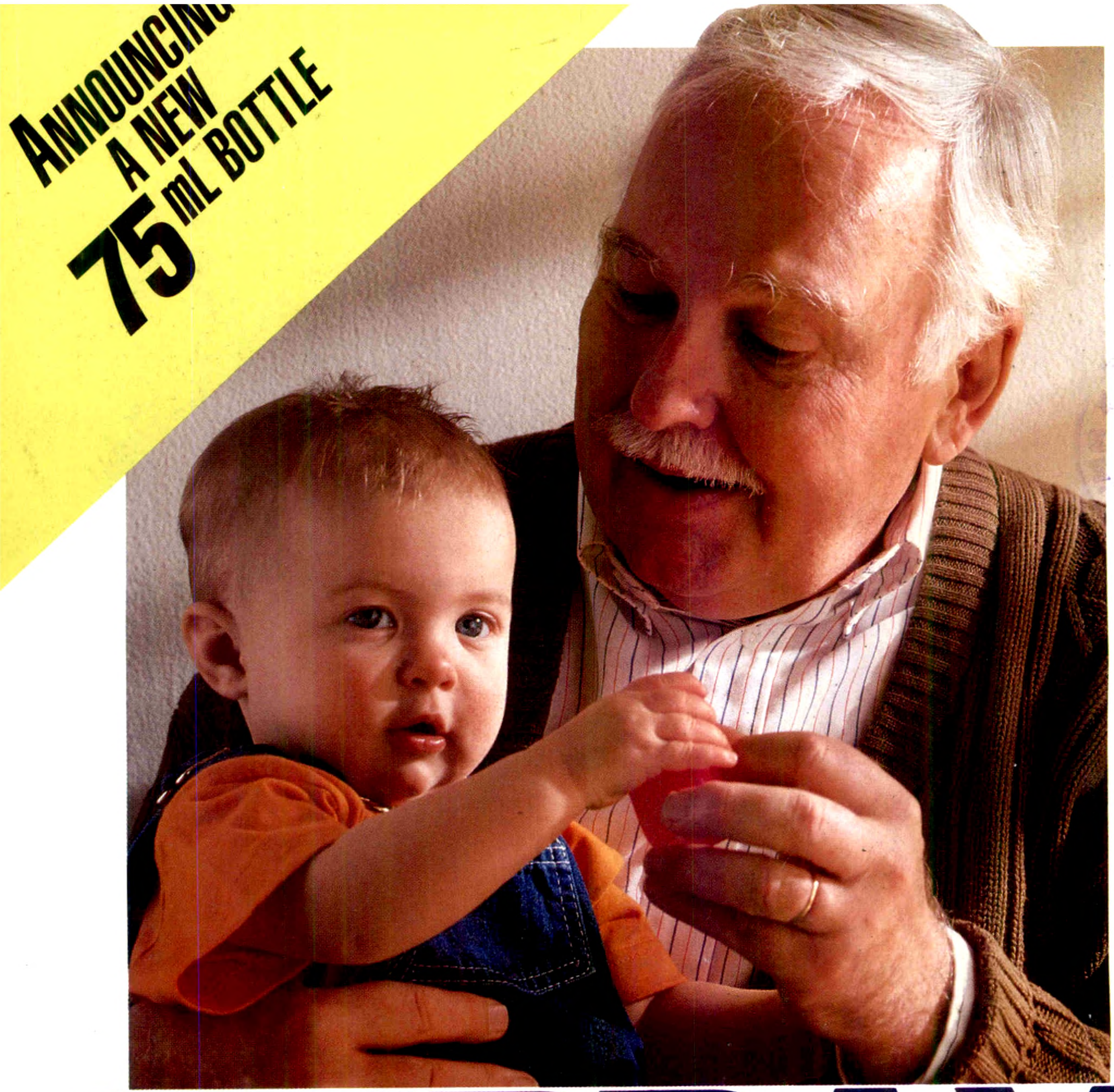

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ANNALS OF HEAD & NECK MEDICINE & SURGERY

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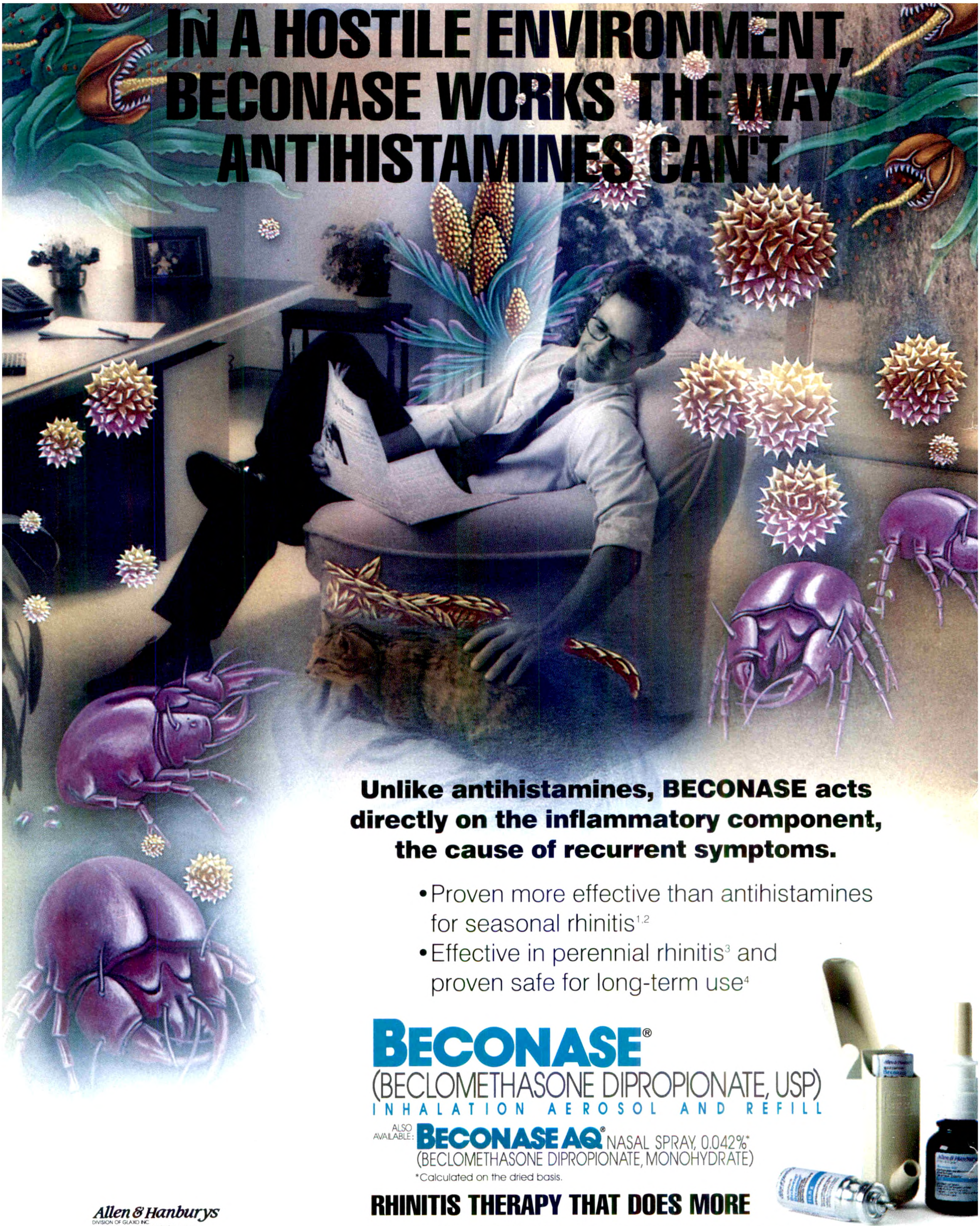

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Inhalation Aerosol
For Nasal Inhalation Only

BRIEF SUMMARY

Beconase AQ®
(beclomethasone dipropionate, monohydrate)
Nasal Spray, 0.042%*
***Calculated on the dried basis.**
For Intranasal Use Only

SHAKE WELL
BEFORE USE.

The following is a brief summary only. Before prescribing, see complete prescribing information in Beconase® Inhalation Aerosol and Beconase AQ® Nasal Spray product labeling.

CONTRAINDICATIONS: Hypersensitivity to any of the ingredients of either preparation contraindicates its use.

WARNINGS: The replacement of a systemic corticosteroid with Beconase® Inhalation Aerosol or Beconase AQ® Nasal Spray can be accompanied by signs of adrenal insufficiency.

Careful attention must be given when patients previously treated for prolonged periods with systemic corticosteroids are transferred to Beconase Inhalation Aerosol or Beconase AQ Nasal Spray. This is particularly important in those patients who have associated asthma or other clinical conditions where too rapid a decrease in systemic corticosteroids may cause a severe exacerbation of their symptoms.

Studies have shown that the combined administration of alternate-day prednisone systemic treatment and orally inhaled beclomethasone increases the likelihood of hypothalamic-pituitary-adrenal (HPA) suppression compared with a therapeutic dose of either one alone. Therefore, Beconase Inhalation Aerosol and Beconase AQ Nasal Spray treatment should be used with caution in patients already on alternate-day prednisone regimens for any disease.

If recommended doses of intranasal beclomethasone are exceeded or if individuals are particularly sensitive or predisposed by virtue of recent systemic steroid therapy, symptoms of hypercorticism may occur, including very rare cases of menstrual irregularities, acneiform lesions, and cushingoid features. If such changes occur, Beconase Inhalation Aerosol and Beconase AQ Nasal Spray should be discontinued slowly consistent with accepted procedures for discontinuing oral steroid therapy.

PRECAUTIONS:

General: During withdrawal from oral steroids, some patients may experience symptoms of withdrawal, e.g., joint and/or muscular pain, lassitude, and depression.

In clinical studies with beclomethasone dipropionate administered intranasally, the development of localized infections of the nose and pharynx with *Candida albicans* has occurred only rarely. When such an infection develops, it may require treatment with appropriate local therapy or discontinuation of treatment with Beconase® Inhalation Aerosol or Beconase AQ Nasal Spray.

Beclomethasone dipropionate is absorbed into the circulation. Use of excessive doses of Beconase Inhalation Aerosol or Beconase AQ Nasal Spray may suppress HPA function.

Beconase Inhalation Aerosol and Beconase AQ Nasal Spray should be used with caution, if at all, in patients with active or quiescent tuberculous infections of the respiratory tract; untreated fungal, bacterial, or systemic viral infections; or ocular herpes simplex.

For either preparation to be effective in the treatment of nasal polyps, the aerosol or spray must be able to enter the nose. Therefore, treatment of nasal polyps with these preparations should be considered adjunctive therapy to surgical removal and/or the use of other medications that will permit effective penetration of these preparations into the nose. Nasal polyps may recur after any form of treatment.

As with any long-term treatment, patients using Beconase Inhalation Aerosol or Beconase AQ Nasal Spray over several months or longer should be examined periodically for possible changes in the nasal mucosa.

Because of the inhibitory effect of corticosteroids on wound healing, patients who have experienced recent nasal septum ulcers, nasal surgery, or trauma should not use a nasal corticosteroid until healing has occurred.

Although systemic effects have been minimal with recommended doses, this potential increases with excessive doses. Therefore, larger than recommended doses of Beconase Inhalation Aerosol and Beconase AQ Nasal Spray should be avoided.

Beconase Inhalation Aerosol: Rare instances of nasal septum perforation have been spontaneously reported.

Rare instances of increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids.

Beconase AQ Nasal Spray: Rarely, immediate hypersensitivity reactions may occur after the intranasal administration of beclomethasone.

Extremely rare instances of wheezing, nasal septum perforation, and increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids. Although these have not been observed in clinical trials with Beconase AQ Nasal Spray, vigilance should be maintained.

If persistent nasopharyngeal irritation occurs, it may be an indication for stopping Beconase AQ Nasal Spray.

Information for Patients: Patients being treated with Beconase Inhalation Aerosol or Beconase AQ Nasal Spray should receive the following information and instructions. This information is intended to aid in the safe and effective use of this medication. It is not a disclosure of all possible adverse or intended effects. Patients should use these preparations at regular intervals since their effectiveness depends on their regular use. The patient should take the medication as directed. It is not acutely effective, and the prescribed dosage should not be increased. Instead, nasal vasoconstrictors or oral antihistamines may be needed until the effects of Beconase Inhalation Aerosol or Beconase AQ Nasal Spray are fully manifested. One to 2 weeks may pass before full relief is obtained. The patient should contact the physician if symptoms do not improve, or if the condition worsens, or if sneezing or nasal irritation occurs. For the proper use of either unit and to attain maximum improvement, the patient should read and follow carefully the patient's instructions section of the package insert.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of rats for a total of 95 weeks, 13 weeks by inhalation and 82 weeks by the oral route, resulted in no evidence of carcinogenic activity. Mutagenic studies have not been performed.

Impairment of fertility, as evidenced by inhibition of the estrous cycle in dogs, was observed following treatment by the oral route. No inhibition of the estrous cycle in dogs was seen following treatment with beclomethasone dipropionate by the inhalation route.

Pregnancy: ***Teratogenic Effects: Pregnancy Category C:*** Like other corticoids, parenteral (subcutaneous) beclomethasone dipropionate has been shown to be teratogenic and embryocidal in the mouse and rabbit when given in doses approximately 10 times the human dose. In these studies, beclomethasone was found to produce fetal resorption, cleft palate, agnathia, microstomia, absence of tongue, delayed ossification, and agenesis of the thymus. No teratogenic or embryocidal effects have been seen in the rat when beclomethasone dipropionate was administered by inhalation at 10 times the human dose or orally at 1,000 times the human dose. There are no adequate and well-controlled studies in pregnant women. Beclomethasone dipropionate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully observed.

Nursing Mothers: It is not known whether beclomethasone dipropionate is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised when Beconase Inhalation Aerosol or Beconase AQ Nasal Spray is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children below 6 years of age have not been established.

ADVERSE REACTIONS: In general, side effects in clinical studies with both preparations have been primarily associated with irritation of the nasal mucous membranes.

Rare cases of immediate and delayed hypersensitivity reactions, including urticaria, angioedema, rash, and bronchospasm, have been reported following the oral and intranasal inhalation and administration of beclomethasone.

Beconase® Inhalation Aerosol: Adverse reactions reported in controlled clinical trials and long-term open studies in patients treated with Beconase Inhalation Aerosol are described below.

Sensations of irritation and burning in the nose (11 per 100 patients) following the use of Beconase Inhalation Aerosol have been reported. Also, occasional sneezing attacks (10 per 100 adult patients) have occurred immediately following the use of the intranasal inhaler. This symptom may be more common in children. Rhinorrhea may occur occasionally (1 per 100 patients).

Localized infections of the nose and pharynx with *Candida albicans* have occurred rarely (see PRECAUTIONS).

Transient episodes of epistaxis have been reported in 2 per 100 patients.

Rare cases of ulceration of the nasal mucosa and instances of nasal septum perforation have been spontaneously reported (see PRECAUTIONS).

Rare instances of increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids (see PRECAUTIONS).

Systemic corticosteroid side effects were not reported during controlled clinical trials. If recommended doses are exceeded, however, or if individuals are particularly sensitive, symptoms of hypercorticism, i.e., Cushing's syndrome, could occur.

Beconase AQ® Nasal Spray: Adverse reactions reported in controlled clinical trials and open studies in patients treated with Beconase AQ Nasal Spray are described below.

Mild nasopharyngeal irritation following the use of beclomethasone aqueous nasal spray has been reported in up to 24% of patients treated, including occasional sneezing attacks (about 4%) occurring immediately following use of the spray. In patients experiencing these symptoms, none had to discontinue treatment. The incidence of transient irritation and sneezing was approximately the same in the group of patients who received placebo in these studies, implying that these complaints may be related to vehicle components of the formulation.

Fewer than 5 per 100 patients reported headache, nausea, or lightheadedness following the use of Beconase AQ Nasal Spray. Fewer than 3 per 100 patients reported nasal stuffiness, nosebleeds, rhinorrhea, or tearing eyes.

Extremely rare instances of wheezing, nasal septum perforation, and increased intraocular pressure have been reported following the intranasal administration of aerosolized corticosteroids (see PRECAUTIONS).

OVERDOSAGE: Information concerning possible overdosage and its treatment appears in the full prescribing information.

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References: 1. Beswick KBJ, Kenyon GS, Cherry JR. A comparative study of beclomethasone dipropionate aqueous nasal spray with terfenadine tablets in seasonal allergic rhinitis. *Curr Med Res Opin.* 1985;9(8):560-567. 2. Salomonsson P, Gottberg L, Heilborn H, Norrlind K, Pegelow K-O. Efficacy of an oral antihistamine, astemizole, as compared to a nasal steroid spray in hay fever. *Allergy.* 1988;43:214-218. 3. Tarlo SM, Cockcroft DW, Dolovich J, Hargreave FE. Beclomethasone dipropionate aerosol in perennial rhinitis. *J Allergy Clin Immunol.* March 1977;59:232-236. 4. Holopainen E, Malmberg H, Binder E. Long-term follow-up of intra-nasal beclomethasone treatment: a clinical and histologic study. *Acta Otolaryngol Suppl (Stockh).* 1982;386:270-273.

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Director: Rodney P. Lusk, MD

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A481

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Languages: English, German.

For further information, contact:

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A484

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Rodney P. Lusk, MD, Director
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For information, contact
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November 4-7, 1992

The Children's Memorial Hospital
Chicago, Illinois
Lauren D. Holinger, MD, Director
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ANNALS OF HEAD & NECK MEDICINE & SURGERY®

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HEARING GAIN AFTER STAPEDOTOMY, PARTIAL PLATINECTOMY, OR TOTAL STAPEDECTOMY FOR OTOSCLEROSIS

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The differences in hearing gain 1 year after stapedotomy, partial platinectomy, or total stapedectomy for otosclerosis were studied separately at 0.5, 1, 2, 4, and 8 kHz with the data available from 311 consecutive primary operations performed between 1980 and 1982 in the University of Nijmegen Department of Otorhinolaryngology. Three groups of patients were matched for age, sex, clinical type of otosclerosis, and type of 0.6-mm all-Teflon piston. The hearing gain for air conduction was significantly better by an average of 7.4 dB for all frequencies combined after either stapedotomy or partial platinectomy compared to total stapedectomy.

KEY WORDS — otosclerosis, stapedotomy, stapes surgery, total stapedectomy.

INTRODUCTION

In 1958 Shea[1] described the operative technique of stapedectomy and introduced the use of a vein graft in conjunction with a polyethylene strut. The stapedectomy procedure has been refined and many modifications have since been introduced. One of these, first advocated by Marquet et al,[2] is to open the footplate only as far as necessary to admit a piston freely. In partial platinectomy a larger part of the footplate is removed. Later reports[3-7] indicate that a limited opening (0.5 to 0.7 mm) of the stapes footplate (stapedotomy) results in a significantly better hearing gain at 4 and 8 kHz. A significantly lower risk of immediate and delayed inner ear deafness is also reported.[3-5] Better results for the stapedotomy technique have also been described by other authors,[8-10] but the reported differences were not significant.

In order to find out whether the mentioned advantages of the stapedotomy technique can be confirmed, the results of the stapedotomy, partial platinectomy, and total stapedectomy techniques with an all-Teflon piston (0.6-mm diameter) obtained in the University of Nijmegen Department of Otorhinolaryngology between 1980 and 1982 were analyzed.

PATIENTS AND METHODS

Between 1980 and 1982, 311 consecutive primary operations for otosclerosis were performed in the Nijmegen department. All ears with a stapes fixation and evidence of tympanosclerosis or a congenital anomaly were excluded. Patients were operated on under local or general anesthesia.

A safety hole was made in the footplate with a thin drill rotating at low speed, as used previously by Causse and Causse[11]; if possible the footplate was opened before removing the stapes suprastructure. The opening of the footplate was usually sealed with blood or Gelfoam. In case of a total stapedectomy, fascia or perichondrium of the tragus was placed over the oval window only occasionally. The operations were performed by eight trained otolaryngologists.

The following variables were used for selection and/or evaluation: age, sex, type of all-Teflon piston used, and clinical type of otosclerosis. Two clinical types of otosclerosis were distinguished. In type 2 otosclerosis, the sensorineural component is equal to or greater than 35 dB at 4 and 8 kHz and the total hearing loss at these frequencies is equal to or greater than 70 dB. Type 1 otosclerosis is the type with threshold values less than the above values at the corresponding frequencies.[12] In these series all-Teflon pistons with a 0.6-mm diameter have mostly been used as replacements for the stapes. The two types of 0.6-mm all-Teflon pistons looked identical, but one was self-manufactured, and the other one was purchased from Smith & Nephew Richards (Memphis, Tenn). Those ears in which pistons of a different material or diameter were used were excluded from the calculations of the hearing gain.

The hearing threshold for air conduction (decibels sound pressure level) was measured at 0.5, 1, 2, 4, and 8 kHz the day before and 6 and 12 months after the operation. The statistics of hearing deterioration were evaluated for the total sample of 311 ears operated on. The whole sample also was used to see whether there was any systematic difference in hearing gain for air conduction at any frequency between follow-up periods of 6 and 12 months. Finally, for a detailed quantitative analysis of the result of hearing, a subsample of 114 ears was

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TABLE 1. HEARING GAIN (DECIBELS) 1 YEAR POSTOPERATIVELY

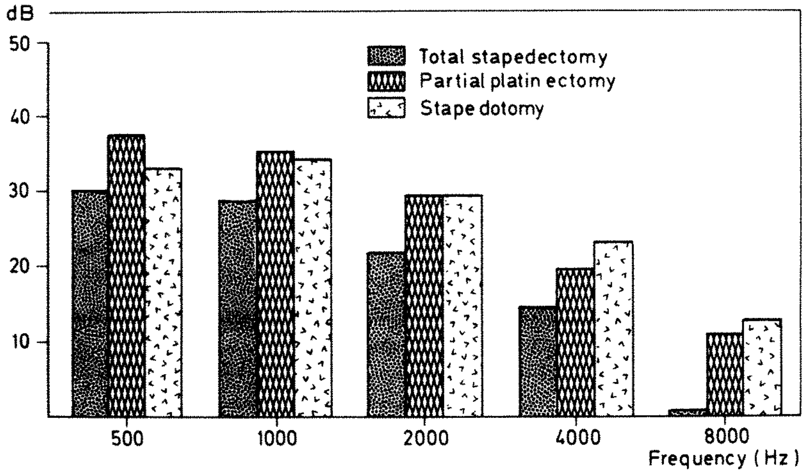
Operative Technique	*Frequency (kHz)* 0.5	1	2	4	8
Total stapedectomy					
No. of observations	30	30	30	29	22
Mean	30.3	28.2	22.0	14.5	0.9
SEM	3.4	3.9	4.3	3.7	3.2
Partial platinectomy					
No. of observations	31	31	31	31	25
Mean	38.1	35.6	29.5	19.8	9.6
SEM	1.9	1.7	2.7	3.4	2.5
Stapedotomy					
No. of observations	31	31	31	30	24
Mean	32.9	34.7	29.4	23.5	12.7
SEM	2.9	2.9	2.9	2.9	2.9
Mean for partial platinectomy and stapedotomy combined	35.5	35.2	29.4	21.6	11.1

selected. This sample was composed of 38 ears for each operative technique, and the patients were carefully matched for sex, age, clinical type of otosclerosis, and the type of 0.6-mm all-Teflon piston used. The threshold shift for air conduction has a normal distribution that allows the use of analysis of variance and Student's *t* test. Significance was defined at p = .05.

RESULTS

In all cases studied, no systematic difference in air conduction improvement was found between 6 and 12 months after the operation. The following quantitative analysis of the hearing result is therefore limited to the 1-year results for the matching groups. The relevant statistics of the hearing gains are presented in Table 1 for the various frequencies and operative techniques. One combination group is included in this Table. The maximum number of observations after 1 year was 31, and some observations are missing, especially at the highest frequencies, because the threshold was beyond the measuring range in the cases concerned. It was determined that no significant difference in the frequency of this event was present between any of the groups (Fisher's exact probability test). The Figure is a graphic illustration of the gain data. A two-way analysis of variance applied to the data of Table 1 (excluding the combination group) showed that there are significant differences that can be attributed to frequency and group factors. The residues after correcting for the differences among the means per group as well as for the means per frequency are smaller than 3.5 dB. Even smaller residues, ie, smaller than 1.5 dB, are found after this procedure has been followed for the 2 x 5 table that results from combining the groups of partial platinectomy and stapedotomy. Student's *t* test was used to compare two groups at a time within Table 1 per frequency in order to detect group effects. At two frequencies only were significant differences detected: at 0.5 kHz between total stapedectomy and partial platinectomy and at 8 kHz between total stapedectomy and each of the other single groups or the combined group. However, a distinct tendency for high *t* values was seen if the total stapedectomy group was tested against either of the two other groups or the combination group. Most of the calculated *t* values correspond to p values in the range of .05 to .20, whereas the hearing gain was always the smallest after total stapedectomy. The fact that at all frequencies Student's *t* test shows a similar tendency suggests that this can hardly be due to chance alone. Table 2 shows that, indeed, there is a constant and significant group difference (Student's *t* test), indicating that the hearing gain after total stapedectomy is on the average 7.4 dB less than in the other groups. It is also clear from Table 2 that there is no systematic difference in hearing gain after stapedotomy and partial platinectomy. Both points can be perceived from the Figure.

The frequencies of postoperative total inner ear deafness and increase of the sensorineural component at 0.5 to 4 kHz by more than 20 dB are presented in Table 3 for all 311 primary consecutive cases. There was no substantial difference among the operative techniques.



Mean hearing gain at each frequency 1 year postoperatively in three groups matched for sex, age, and clinical type of otosclerosis. There is no systematic difference in gain after stapedotomy or partial platinectomy, but gain of either of these groups is systematically better at all frequencies than gain after total stapedectomy by average of 7.4 dB. This effect is significant (see Table 2).

TABLE 2. DIFFERENCES IN MEAN HEARING GAIN (DECIBELS) BY OPERATIVE TECHNIQUE

	Frequency (kHz)						
Operative Technique	*0.5*	*1*	*2*	*4*	*8*	*Mean*	*SD*
Partial platinectomy minus total stapedectomy	7.7	7.5	7.5	5.4	8.7	7.4	1.2
Stapedotomy minus total stapedectomy	2.6	6.5	7.4	9.0	11.8	7.4	3.4
Stapedotomy and partial platinectomy minus total stapedectomy	5.2	7.0	7.4	7.2	10.2	7.4	1.8
Stapedotomy minus partial platinectomy	5.2	1.0	0.2	−3.7	− 3.1	0.1	3.6

DISCUSSION

The obtained results indicate better gains of hearing at all frequencies for stapedotomy and partial platinectomy compared to total stapedectomy. Until now significantly better results have only been reported at 4 and 8 kHz for stapedotomy compared to total stapedectomy.[3-7] The reported differences below 4 kHz were neither consistent nor significant.

Teflon wire and stainless steel are the prosthesis materials most studied.[5,7,9,12] All-Teflon pistons have been used only by Marquet et al[2] (0.6-mm diameter) and Smyth et al[3,5] (0.3- and 0.8-mm diameter); the diameter of the all-Teflon piston used in this study was 0.6 mm. The pistons used by Marquet et al[2] are also manufactured in the same workshop at Nijmegen and are exactly the same as one of the types used in this study (J. Marquet, personal communication, 1985). The diameter of the piston can probably influence the result. An all-Teflon piston with a 0.3-mm diameter has proven not to be optimal.[3,4] The greater the diameter of the prosthesis, the larger the opening in the footplate has to be. Widening a small hole (0.5 mm) can eventually lead to a subtotal platinectomy, particularly in the presence of minimal otosclerotic fixation, which then prohibits a limited footplate fenestration. Fisch[4,13] therefore adapted his technique of stapedotomy and he prefers a 0.4-mm Teflon-wire piston.

The relative incidences of postoperative total inner ear deafness or increase of the sensorineural component at 0.5 to 4 kHz by more than 20 dB, as presented in Table 3, correspond with those in the literature.

The obtained results are surprising since they not only confirm the better results of stapedotomy and partial platinectomy compared to total stapedectomy at 4 and 8 kHz, but extend this also to 0.5, 1, and 2 kHz. Techniques or procedures that will prove to be the most successful in avoiding total stapedectomies are therefore preferable. However, if stapedotomy or partial platinectomy is unfeasible, a total stapedectomy still remains a good alternative from the viewpoint of the probable hearing gain and the relatively low risk of postoperative damage to the inner ear.

TABLE 3. NUMBERS OF EARS WITH INCREASED SENSORINEURAL HEARING LOSS 1 YEAR POSTOPERATIVELY

		Total Deafness		*Loss Increased by 20 dB at 0.5 to 4 kHz*	
Operative Technique	*No.*	*N*	*%*	*N*	*%*
Total stapedectomy	48	1	2.1	1	2.1
Partial platinectomy	102	0	0.0	0	0.0
Stapedotomy	161	1	0.6	1	0.6
Total	311	2	0.6	2	0.6

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BLOOD FLOW IN EXPANDED TISSUE TREATED WITH AN ANTICONTRACTILE AGENT

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IOWA CITY, IOWA

The present study investigates blood flow at several stages of expansion with and without enhancement by anticontractile agents (papaverine). Twelve 1-kg guinea pigs were divided into two groups. In group 1 were six animals undergoing tissue expansion without anticontractile agents, and in group 2 were six animals treated with a saturated solution of papaverine prior to expansion. Laser-Doppler velocimetry was used to monitor the changes in blood perfusion that occurred with each expansion. Animals pretreated with papaverine (group 2) maintained higher blood flow following inflation of the expander than untreated animals (group 1). This study provides physiologic evidence of the benefits of pharmacologic enhancement of the expansion process. Agents of this type may have a future role in the prevention of ischemic complications.

KEY WORDS — laser-Doppler velocimetry, muscle relaxant, tissue expander.

INTRODUCTION

Tissue expansion is being increasingly used by surgeons in a wide variety of cases. However, in reviewing the literature, one notes that clinical acceptance of tissue expansion has occurred much faster than basic research has accumulated. While there have been a few experimental studies of tissue expansion, most of these emphasize structural rather than functional parameters.[1-3] Histologic studies have shown that the thickness of the epidermis covering the expander is preserved by an increase in the mitotic activity of the expanding epidermis.[1,2,4] However, as expansion occurs the tissue layer adjacent to the expander becomes organized into a fibrous capsule.[5] Ultrastructural studies have demonstrated that the cellular components of the capsule include contractile fibroblasts, which are modified fibroblasts containing actin and myosin. These cells are believed to be responsible for the contraction of the capsule surrounding the tissue expander as well as for the contraction of healing wounds.[6] Recent studies from our laboratory[7-9] have shown that smooth muscle relaxants such as cytochalasin, theophylline, and papaverine reduce the contractile effect of the contractile fibroblasts and thus enhance tissue expansion.

Although much has been learned about the morphology of expanded tissue, very little is known about changes in functional parameters such as blood flow that occur during and after expansion. Even less is known about alterations of blood flow that might follow treatment of expanded tissue with smooth muscle relaxant. This is clearly of importance for maintaining adequate perfusion at the time of skin expansion and subsequent use of the skin flap to ensure flap survival.[10] Measuring changes in blood flowing is of special value in cases of accelerated tissue expansion with immediate use of skin flaps.

The present study was designed to investigate blood perfusion at several stages of expansion with and without enhancement by anticontractile agents. To learn more about blood flow at the time of tissue expansion, we compared changes following routine expansion with those following expansion enhanced by the use of smooth muscle relaxants.

MATERIALS AND METHODS

Twelve 1-kg guinea pigs were divided into two groups. Group 1 consisted of six animals that underwent a standard expansion using 50-mL hemispherical expanders without anticontractile agents. Group 2 consisted of six animals that were expanded at the same rate as group 1 animals, but prior to each inflation, this group received pretreatment with 1.5 mL of a saturated solution of papaverine hydrochloride. Papaverine was selected because of its documented ability to facilitate expansion and its low incidence of side effects. The concentration used (20 mg/mL) represents that which has been previously shown to exert an effect on contractile cells.[7] The papaverine was administered at the base of the expander by means of the delivery system described below.

The guinea pigs were maintained in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Re-

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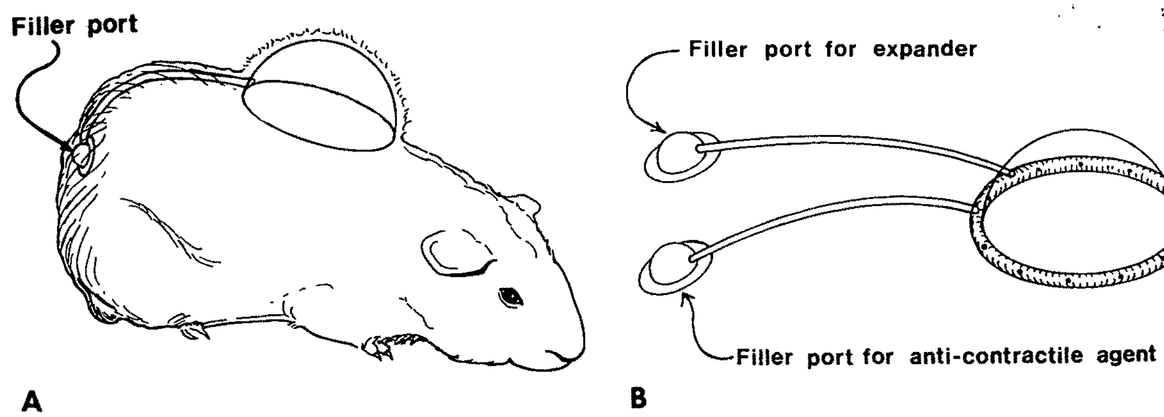
From the Department of Otolaryngology–Head and Neck Surgery (Canady, Kelly, Bardach) and the Dows Institute for Dental Research (Squier), The University of Iowa, Iowa City, Iowa. Support for this research was provided in part by Public Health Service Research Grant BR56-2-S07-RR-5313 and a gift of tissue expanders from the Dow Corning Company. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Iowa.

REPRINTS — John W. Canady, MD, Dept of Otolaryngology–Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242.



Expander dome. A) In place and filled. B) Modified to allow introduction of anticontractile agent around dome.

search as well as the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Science. The animals were fed a standard laboratory diet and water ad libitum and housed singly in cages.

Group 1 animals were implanted with commercially available 50-mL hemispherical expanders donated for this project by the Dow Corning Company, Midland, Mich (see Figure, A). The expanders implanted in group 2 animals were modified to allow even distribution of the papaverine around the expander (see Figure, B). Specifically, a small Silastic tube that was sealed at one end was attached circumferentially around the base of the expander dome with silicone adhesive. The distal 10 cm of this tube surrounding the expander base was perforated with 16 small holes to allow even distribution of the anticontractile agent around the expander. The papaverine solution was injected prior to each inflation via a separate port attached to the Silastic tube.

All operations were performed under sterile conditions with the animals anesthetized with Ketaset (ketamine hydrochloride, 30 mg/kg) and Rompun (xylazine, 3 mg/kg). Implantation of the expanders was carried out deep to the panniculus carnosus into a subcutaneous tunnel beginning at the sacrum and ending at the intraspinous area between the scapular spines. The injection ports were placed in a separate subcutaneous pocket caudally over the sacrum. The incisions were closed with 3-0 chromic suture. At the time of implantation, 5 mL of saline was injected into each expander to decrease the amount of dead space and to help stabilize the expander, thus preventing seroma formation.

The animals were allowed to heal for 3 days before inflation was begun. The amount and frequency at which the expanders were inflated did not vary between the groups. The expanders were filled every 3 days with isotonic saline. Ten percent of the final expander volume, or 5 mL, was added at each interval. This volume of injected saline caused the overlying skin to become noticeably taut; this is similar to the criterion used to determine the filling rate during normal clinical application in humans.[11-13] Expansion was continued until the end volume of 25 mL had been injected into each expander. This value was chosen because, in relation to overall body size of the guinea pig, it represents greater inflation than would likely be employed in a human. This is also the point at which systemic complications such as compromised respiration and decreased locomotion began to greatly influence the health and comfort of the animals.

Blood flow in the expanded tissue was monitored by using laser-Doppler velocimetry (LDV). Laser-Doppler velocimetry provides a noninvasive means of measuring blood flow without damage to the tissue being examined. Furthermore, since the tissue remains intact and viable, events can be followed over time.[14-16] The principle involved in this technique resembles the Doppler shift phenomenon that occurs in a moving sound source, but in LDV it is photons from a laser source that are scattered and shifted by moving red blood cells. Plasma surrounding the red blood cells does not cause a shift of the light and therefore will not influence the readings. Some of the reflected photons are recovered via optical fibers in the transducer head housing the laser and are conveyed to a photodetector. At this point the light is converted to an electrical signal that can be passed through a microprocessor and expressed as values that are proportional to blood flow, volume, or velocity.

During each expansion and measurement, the animals were sedated with ketamine hydrochloride (15 to 25 mg/kg) to prevent extraneous movement that would interfere with the readings of blood flow that were to be made with the laser-Doppler unit. The laser-Doppler unit used in this study (Laserflo model BPM 403 blood perfusion monitor, loaned by TSI, Minneapolis, Minn) illuminates a 1.5-mm^3 volume of tissue beneath the transducer head. Various averaging times are available to compensate for movement of the animal or the transducer. In the present study, an averaging time of 5 seconds

PERCENT DROP IN BLOOD FLOW AS MEASURED BY LASER-DOPPLER VELOCIMETRY

	Amount of Expansion			
Treatment Group	*5 mL to 10 mL*	*10 mL to 15 mL*	*15 mL to 20 mL*	*20 mL to 25 mL*
No relaxant	57 (± 4)	49 (±14)	37 (±11)	25 (± 9)
Papaverine	33 (±10)	34 (±17)	14 (± 6)	3 (±10)

Values represent group means (±95% confidence interval).

was used for all the readings. The values obtained by LDV are relative values expressed in millivolts. Blood flow readings were taken immediately adjacent to the central point of the expanded tissue, which was tattooed for reference. The measurement sites were shaved on the day prior to each inflation so that local irritation from the shaving would not influence flow readings.

Laser-Doppler velocimetry has proven to be most useful for continuous monitoring of blood perfusion.[17,18] However, the small sampling volume (1.5 mm^3) makes it difficult to resample the same volume of tissue once the transducer has been moved, so that a sampling error can be introduced in attempts to monitor blood flow over time. In this study, readings were taken before and after each separate inflation in both treated and untreated groups. All blood flow readings were taken immediately adjacent to the central point of the expanded tissue, which was marked with a tattoo. In order to ensure comparability between measurements recorded before and after inflation, the transducer head remained firmly affixed adjacent to the tattoo mark throughout the measuring period by means of an adhesive ring supplied by the manufacturer. The LDV readings were examined by using an analysis of variance to determine significant difference.

RESULTS

The Table presents the changes in blood flow in animals undergoing expansion facilitated by papaverine (group 2) as compared with animals expanded without pharmacologic mediators (ie, "routine" expansion; group 1). In order to facilitate comparisons between the groups, the average change in blood flow from before to after inflation within each group is expressed for each of four consecutive increases in expander volume. The blood flow values represent the percentage decline following each inflation from the preexpansion baseline values.

Analysis of the blood flow data demonstrated that for every filling except that from 10 mL to 15 mL, the reduction in blood flow after inflation was significantly less ($p < .05$) in the group treated with anticontractile agents (group 2). Also, within each group there was significantly less change between the preexpansion and postexpansion blood flow when the expanders were filled from 20 mL to 25 mL compared to when they were filled from 5 mL to 10 mL ($p < .05$). When the values for each inflation were compared by using analysis of variance with interaction between time and blood flow, it was found that each inflation period (5 mL to 10 mL, 10 mL to 15 mL, etc) had a significantly different recovery of blood flow ($p < .05$).

DISCUSSION

In the present study, pretreatment with papaverine (group 2) maintained a higher postexpansion blood flow than that seen in the group expanded at the same rate but without pharmacologic intervention (group 1). This may be the result not only of relaxation of the myofibroblasts in the capsule by papaverine, but also of local drug effects on the vasculature leading to vasodilation and an increase in perfusion. Regardless of the exact site of action, the data suggest that pharmacologically enhanced expansion may hold added benefits when compared to simple expansion alone. Agents such as papaverine may increase the safety margin and reduce the risks of ischemic complications during expansion. As the expansion period progressed, there was less change between preexpansion and postexpansion blood flow in the treated and untreated groups. Whether this finding is due to an adaptive process resulting from formation of additional skin vessels or to contributions from a developing capsular blood flow is as yet unknown, but it may help explain the observation that flaps raised on expanded skin have been shown to have increased survival much as though they had been delayed in a more traditional fashion.

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ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

The 1992 Midwinter Meeting of the Association for Research in Otolaryngology will be held February 2-6, 1992, at the Tradewinds Hotel, St Petersburg Beach, Florida. For further information, contact Michele Johnson, Executive Secretary, 900 Des Moines Street, Suite 200, Des Moines, IA 50309; Phone (515) 266-2189, Fax (515) 266-0613.

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DIAGNOSTIC AND OPERATIVE SINUS ENDOSCOPY

A course on Diagnostic and Operative Sinus Endoscopy will be held January 24-25, 1992, at the NYU Medical Center, Schwartz Lecture Hall, in New York. For further information, contact NYU Medical Center, Post-Graduate Medical School, 550 First Avenue, New York, NY 10016; (212) 263-5295.

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UTRICLE, SACCULE, AND COCHLEAR DUCT IN RELATION TO STAPEDOTOMY

A HISTOLOGIC HUMAN TEMPORAL BONE STUDY

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This study was performed to determine the area in which and the circumstances under which stapedotomy can be relatively safely performed. Measurements were made from central areas of the medial surface of the stapedial footplate to the utricle, the saccule, and the cochlear duct in 10 normal and 11 otosclerotic temporal bones. The mean distances to the utricle ranged from 1.9 to 2.4 mm, and those to the saccule from 1.7 to 2.1 mm. The minimal distance to the utricle was measured from the posterior (0.58 mm) and superior (0.62 mm) borders of the stapedial footplate. The minimal distances to the saccule were from the anterior (0.76, 0.86, and 1.00 mm) border of the stapedial footplate. All other measurements were of more than 1 mm. The shortest distance between the cochlear duct and the inferior border of the footplate was 0.2 mm. Statistical analysis has shown no significant differences for the mean values obtained in normal and otosclerotic temporal bones. Fathoming of the vestibule below the central and inferior thirds of the footplate surface has shown that there is no likely danger to the vestibular end organs or cochlear duct if manipulations are carried out no deeper than 1 mm below the surface. The safest place for a stapedotomy opening is in the central and inferior-central thirds of the footplate. A stapedotomy piston of 0.4 mm in diameter can be introduced relatively safely to a depth of 0.5 mm in the vestibule over the entire surface of the stapedial footplate.

KEY WORDS — cochlea, ear ossicles, otosclerosis, saccule, stapes surgery, temporal bone, utricle, vestibule.

INTRODUCTION

Early attempts to improve hearing loss caused by otosclerosis stimulated several studies dealing with the relationship between the base of the stapes and the vestibular end organ.[1-6] These resulted in data providing more insight into the dangers of mobilizing and removing the whole stapedial footplate. Most of the known data are related to the removal of the complete footplate (stapedectomy) and therefore concern measurements from the border of the stapedial footplate or oval window. Only Anson and Donaldson[3] made measurements at a level passing through the maximum width of the footplate. When a limited opening of the footplate is used, as in stapedotomy,[7-9] the distances between the inner ear structures and the more central area of the footplate are of primary importance. Therefore, in this study, measurements were taken from a central 4-mm² area of the footplate to the utricle, the saccule, and the cochlear duct in normal and otosclerotic temporal bones.

MATERIAL AND METHODS

Serially sectioned human temporal bones from the collection of the Department of Otolaryngology at University Hospital Zürich were used. Ten temporal bones were normal (seven males and three females; mean age, 51 years; range, 4 to 97 years); 11 temporal bones were from patients with otosclerosis (six males and five females; mean age, 67 years; range, 43 to 88 years). The extent of otosclerotic involvement of the footplate was classified according to Gristwood and Venables,[10] as follows: minor degree (five cases), intermediate degree (four cases), and severe degree (two cases).

The temporal bones were fixed in 4% formaldehyde. After decalcification in 5% trichloroacetic acid, they were embedded in celloidin and sectioned in a horizontal plane at a 20-μm thickness. Every

Fig 1. Stapes footplate showing planes and localization of points from which measurements were taken. P — posterior border, PC — 0.2 mm posterior to middle, C — midway, AC — 0.2 mm anterior to middle, A — anterior border, S — superior border, I — inferior border.

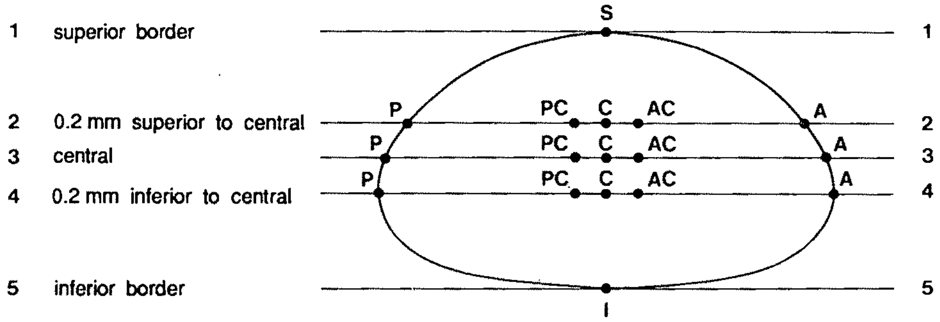

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TABLE 1. RESULTS OF MEASUREMENTS IN NORMAL TEMPORAL BONES

Planes	*Measuring Points*	*Utricle*					*Saccule*				
		n	*Min*	*Max*	*Mean*	*SD*	*n*	*Min*	*Max*	*Mean*	*SD*
Superior		10	0.62	2.10	1.30	0.48	10	1.19	1.76	1.51	0.19
0.2 mm superocentral	P	9	0.58	2.00	1.56	0.44	9	2.19	3.10	2.52	0.29
	PC	9	1.31	2.62	1.91	0.40	9	1.66	2.29	1.96	0.20
	C	9	1.47	2.71	2.00	0.39	9	1.61	2.14	1.82	0.18
	AC	9	1.54	2.81	2.11	0.38	9	1.56	1.95	1.73	0.14
	A	9	2.05	3.43	2.72	0.40	9	0.76	1.50	1.27	0.21
Central	P	10	1.29	2.16	1.70	0.28	10	2.29	3.05	2.66	0.22
	PC	10	1.59	2.81	2.06	0.37	10	1.70	2.24	2.03	0.16
	C	10	1.68	2.95	2.15	0.39	10	1.63	2.16	1.93	0.15
	AC	10	1.77	3.10	2.25	0.40	10	1.61	2.05	1.82	0.14
	A	10	2.38	3.57	2.82	0.41	10	0.86	1.68	1.32	0.24
0.2 mm inferocentral	P	10	1.38	2.43	1.77	0.34	10	2.18	2.95	2.59	0.26
	PC	10	1.54	3.00	2.08	0.47	10	1.93	2.30	2.13	0.14
	C	10	1.61	31.4	2.19	0.47	10	1.82	2.19	2.03	0.15
	AC	10	1.73	3.24	2.29	0.48	10	1.73	2.10	1.92	0.14
	A	10	2.14	4.05	2.88	0.58	10	1.00	1.86	1.50	0.30
Inferior		10	1.67	2.95	2.20	0.39	10	2.05	2.71	2.40	0.18

Measurements are in millimeters and show distances to utricle and saccule from points on medial surface of stapes footplate.
P — posterior border, PC — 0.2 mm posterior to middle, C — midway, AC — 0.2 mm anterior to middle, A — anterior border.

10th section was stained with hematoxylin and eosin. At the time of cutting, the same plane of section was routinely used for each specimen. Small differences in the angle of cutting were considered negligible in this study.

The shortest distances from the medial surface of the stapedial footplate to the utricle and saccule were measured from five horizontal planes situated at the following levels.

1. Tangential to the most superior border of the footplate.
2. Parallel and 0.2 mm superior to the central plane.
3. Through the center of the footplate (ie, halfway between the superior and inferior borders of the footplate).
4. Parallel and 0.2 mm inferior to the central plane.
5. Tangential to the most inferior border of the footplate.

Measurements were taken from the following points at the medial surface of the footplate along three of the above-mentioned planes, namely along the center as well as along those points situated 0.2 mm superior and 0.2 mm inferior to it (Fig 1).

1. At the intersection with the anterior border (A).
2. At the intersection with the posterior border (P).
3. Midway between the posterior and anterior borders (C).
4. At a point 0.2 mm anterior to the middle (AC).
5. At a point 0.2 mm posterior to the middle (PC).

The choice of a 0.4-mm-wide central area of the footplate was determined by the current use of a 0.4-mm stapedotomy platinum-Teflon piston (Xomed-Treace) in our department. When the distance between the superior border of the footplate and the utricle or saccule was smaller than 1 mm, measurements were taken in 200-μm steps at lower levels until the range of 1 mm was reached again. The same procedure was adopted when the distance measured from point A or P was found to be less than 1 mm. Since the cochlear duct lies close to the inferior border of the footplate, the distance between these two structures was also determined by using histologic sections cut below the inferior border of the footplate.

All measurements were made with an ocular micrometer at x25 magnification. The *t* test was used to analyze the statistical significance of the obtained data.

RESULTS

Tables 1 and 2 show the mean, standard deviation, and range of the distances measured between the medial surface of the footplate and the stapes, utricle, and saccule. No statistical difference was found between measurements taken in normal and otosclerotic temporal bones.

Mean Values. The shortest mean distance between the utricle and footplate was measured along the posterior border (point P); the largest mean distance was at the anterior border (point A) of the stapedial footplate (Fig 2A,B). The reverse was true for the saccule, which is closer to the anterior than to the posterior border of the stapes footplate (Fig 2C,D). The distances measured from the central area of the footplate (points AC and PC) to the utricle ranged between 1.9 and 2.4 mm, and those to the saccule, between 1.7 and 2.1 mm.

Minimal Values. The minimal values are most important for surgical purposes, since they determine the safety margin for manipulations within the vestibule. The minimal distance from the utricle to the medial surface of the footplate was measured in normal bones at the posterior (0.58 mm) and su-

TABLE 2. RESULTS OF MEASUREMENTS IN TEMPORAL BONES WITH OTOSCLEROSIS

Planes	Measuring Points	Utricle					Saccule				
		n	*Min*	*Max*	*Mean*	*SD*	*n*	*Min*	*Max*	*Mean*	*SD*
Superior		9	0.71	2.48	1.22	0.61	9	1.00	1.86	1.38	0.29
0.2 mm superocentral	P	9	0.91	2.76	1.67	0.68	9	1.62	3.14	2.38	0.58
	PC	9	1.29	2.95	2.09	0.68	9	1.57	2.38	1.88	0.30
	C	9	1.43	3.10	2.22	0.69	9	1.48	2.24	1.79	0.27
	AC	9	1.52	3.19	2.31	0.63	9	1.38	2.14	1.72	0.26
	A	9	2.33	3.76	2.95	0.49	9	1.00	1.57	1.40	0.20
Central	P	10	1.14	2.81	1.82	0.52	10	2.05	3.14	2.59	0.38
	PC	10	1.24	3.14	2.18	0.62	10	1.67	2.48	1.95	0.28
	C	10	1.33	3.24	2.27	0.64	10	1.48	2.38	1.86	0.29
	AC	10	1.57	3.29	2.35	0.60	10	1.38	2.24	1.74	0.29
	A	10	2.48	3.67	3.06	0.51	10	0.91	1.81	1.37	0.28
0.2 mm inferocentral	P	9	1.14	2.81	1.90	0.53	9	1.71	3.40	2.60	0.54
	PC	9	1.29	3.00	2.23	0.52	9	1.81	2.62	2.13	0.30
	C	9	1.33	3.10	2.35	0.56	9	1.71	2.48	2.00	0.23
	AC	9	1.43	3.14	2.43	0.55	9	1.62	2.38	1.92	0.23
	A	9	2.38	3.57	3.05	0.46	9	1.05	2.10	1.57	0.29
Inferior		7	1.43	2.86	2.16	0.45	10	1.95	2.71	2.26	0.25

Measurements are in millimeters and show distances to utricle and saccule from points on medial surface of stapes footplate.
Abbreviations as in Table 1.

perior (0.62 mm) borders of the stapes (Table 1 and Fig 2). The shortest measurements to the saccule were obtained in normal temporal bones at the anterior border of the footplate (0.76, 0.86, and 1.00 mm; Table 1 and Fig 2).

All other measurements between the medial surface of the footplate and the utricle and saccule were of more than 1 mm. Therefore, manipulation in the vestibule of less than 1 mm can be performed relatively safely in the central and inferior thirds of the footplate (Fig 3A). A minimal distance of less than 0.75 mm was only found in three temporal bones: 0.2 mm below the superior border of the footplate in one normal and one otosclerotic temporal bone and 0.24 mm anterior to the posterior border of the footplate in one normal temporal bone (Fig 3B). The utricle and saccule were found at a distance of more than 0.5 mm from any part of the medial surface of the stapes footplate (Fig 3C).

Measurements taken from the inferior border of the footplate have shown that the cochlear duct was situated inferior to this point in all temporal bones. The mean distance between the cochlear duct and the inferior border of the footplate was 0.6 mm (range, 0.2 to 1.0 mm) in normal bones and 0.4 mm (range, 0.2 to 0.8 mm) in otosclerotic bones.

DISCUSSION

The mean values of the distance from the medial surface of the stapes footplate to the utricle and saccule do not show significant differences in normal and otosclerotic temporal bones. The shortest distances to the utricle were measured in two normal temporal bones along the superior (0.62 mm) and posterior (0.58 mm) borders of the footplate. The shortest distance to the saccule (0.76 mm) also occurred at the anterior border in one normal temporal bone. The measurements taken at the inferior border of the stapedial footplate have shown that the cochlear duct is more frequently found close to it (0.2 mm) in the presence of otosclerosis (four cases) than in normal temporal bones (one case). However, the position of the cochlear duct is always inferior to the lowest border of the stapes footplate, so danger of a lesion to it probably does not exist if a stapedotomy is performed in the central area of the footplate.

The minimal distances to the utricle and saccule obtained in this study from the borders of the footplate confirm those published by other authors.[1,2,4,6] Smaller values of 0.2 to 0.9 mm (mean, 0.5 mm) to the utricle measured at the posterior-superior border of the footplate were reported by Leonard and Alexander[5] using 200 normal temporal bones (100 pairs) cut in a vertical plane. Only seven specimens were found to have a shortest distance to the utricle of 0.3 mm, and only one of 0.2 mm; no measurements to the saccule were made. In a representative otologic series, Anson and Donaldson[3] found, at a level passing through the footplate at its maximum width, a shortest distance to the utricle and saccule of 1.4 mm.

Differences in measurements from histologic material may result from shrinkage due to fixation. According to Igarashi et al,[11] the tissue shrinkage secondary to fixation with 10% formalin, decalcification with 5% trichloroacetic solution, and embedding in celloidin is 9.2% to 9.3%. An error of this magnitude has to be accepted when relating histologic studies to the in vivo situation.

The interest of previous authors was limited to the distances between the vestibular end organ and the margin of the footplate, since at their time the complete removal of the stapes (stapedectomy) was the current surgical procedure for otosclerosis. In

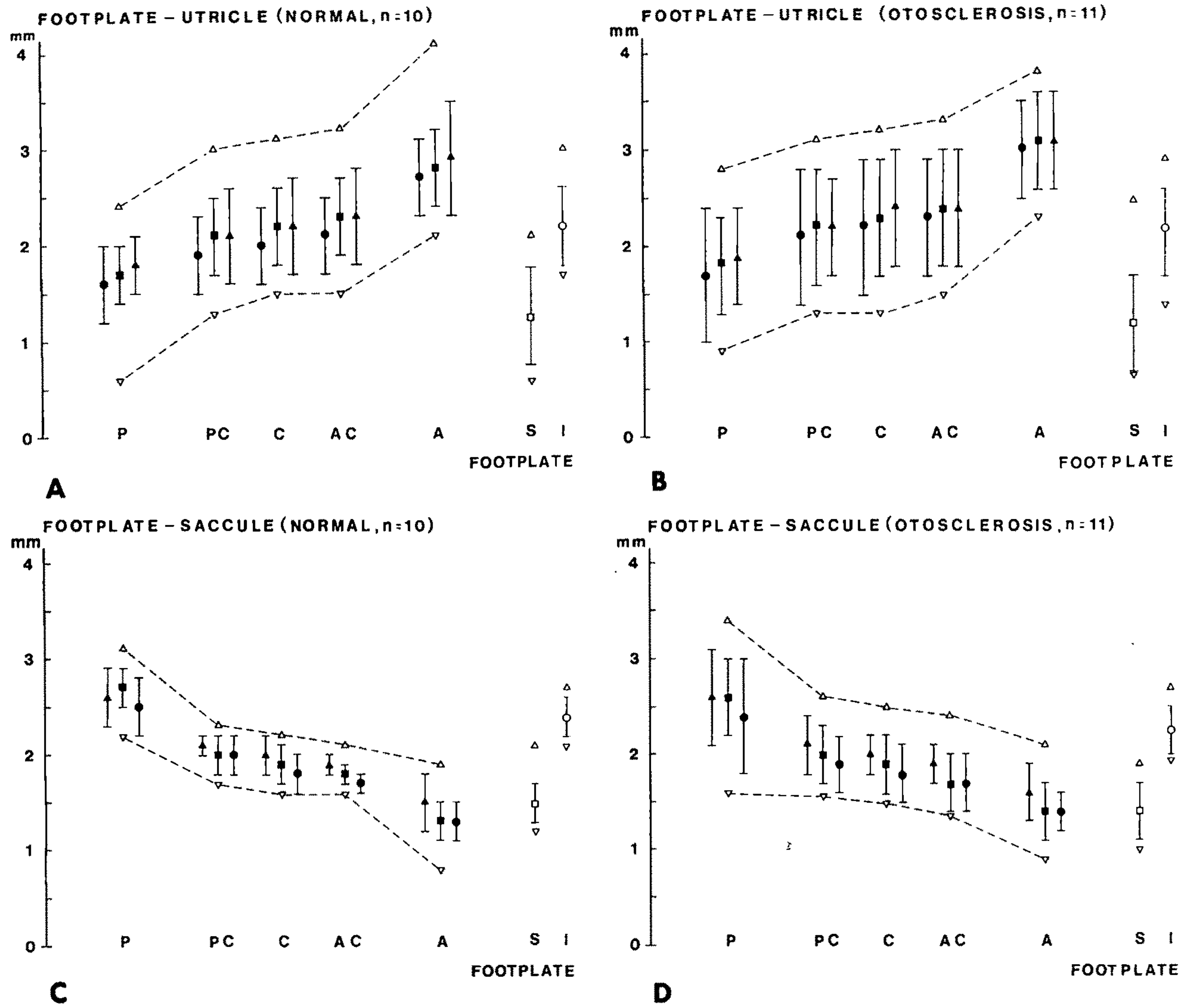

Fig 2. Means, SDs, and minimal and maximal values (dotted lines) of distances between footplate and A,B) utricle and C,D) saccule. A,C) Normal. B,D) Otosclerosis. □ — superior border, ● — 0.2 mm superior to center, ■ — center, ▲ — 0.2 mm inferior to center, ○ — inferior border, ▽ — minimal distance, △ — maximal distance.

view of the widening use of a limited opening in more central areas of the footplate (stapedotomy), fathoming of the safety depth for manipulations in these regions was appropriate.

Our results show that at a 1-mm depth the danger of injury to the utricle existed in 1 of 3 temporal bones (8 of 21; 4 normal, 4 otosclerotic) in the upper third of the footplate, as well as in 1 of 7 (3 of 21) specimens toward the posterior border of the stapes. At the same depth of 1 mm, the lesion of the saccule would have occurred in 1 of 10 (2 of 21) temporal bones. Therefore, the "safe" area for intravestibular manipulations at the 1-mm level extends to the central and inferior thirds of the stapedial footplate (Fig 3A). At a depth of 0.75 mm the only "unsafe" areas of the footplate are its most superior and posterosuperior margins (Fig 3B). No vestibular end organs are endangered under the entire surface of the footplate if the manipulations in the vestibule do not exceed a depth of 0.5 mm (Fig 3C).

The presented data are relevant to performing stapedotomy in otosclerotic ears. The central region of the footplate is the safest place for a limited opening in the footplate. The minimal distance from the footplate center to the utricle is 1.2 mm, and to the saccule, 1.4 mm. The use of perforating instruments in the center of the footplate is relatively safe, if the tip of the perforators is not introduced more than 0.5 mm into the vestibule. The possible intravestibular length of a piston is also critical for good results in stapes surgery. Our clinical experience[8,9] has shown that if a piston is introduced only 0.2 to 0.3 mm through a stapedotomy opening — as proposed by some authors[12] — its accidental dislocation out of the vestibule may occur during further manipulation of the malleus and incus or from spontaneous movements of the remaining ossicular chain (eg, during sneezing). Hüttenbrink[13] found that the piston displacement caused in human temporal bones by varying static air pressure from 0 to 400 mm H_2O can exceed 0.5 mm if the incudomalleal joint is ankylosed. Our clinical observation has also shown that a piston protruding only 0.2 to 0.3 mm into the vestibule can be dislocated by attacks of sneezing or larger external pressure changes (eg, flying). This was the reason why a protrusion of 0.5 mm within the vestibule from the central area of the footplate has been practiced in our department for 10 years in over 1,500 stapedotomies, without apparent lesion of the vestibular end organ or cochlear duct.[8,9]

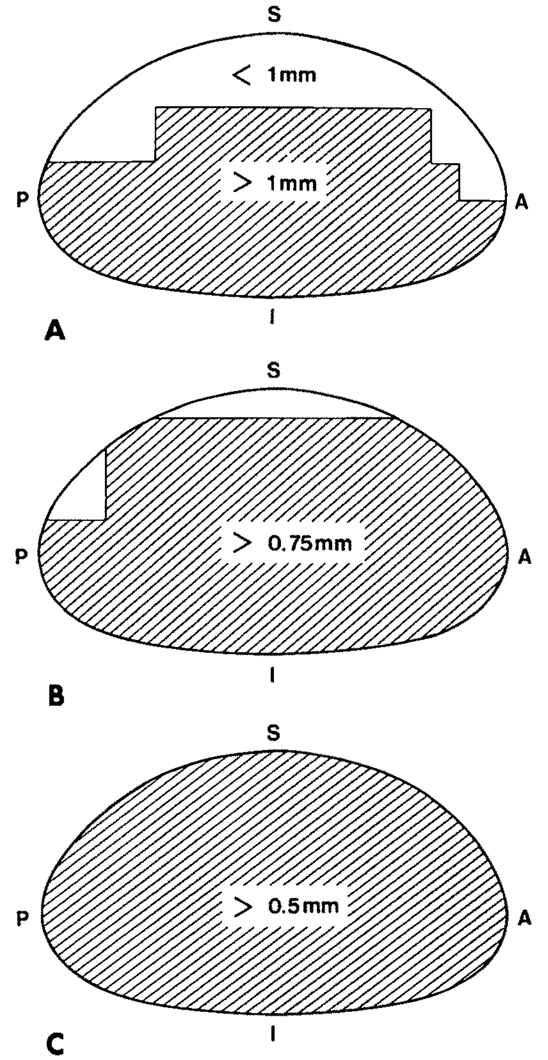

Fig 3. Stapes footplate showing area (striped) with minimal distance between footplate and utricle or saccule of A) >1 mm, B) >0.75 mm, and C) >0.5 mm. P — posterior, A — anterior, S — superior, I — inferior.

CONCLUSIONS

1. The statistical analysis of measurements taken from the medial surface of the stapedial footplate to the vestibular end organs and cochlear duct has shown no significant differences for the mean values obtained in normal and otosclerotic temporal bones.

2. The minimal distances to the vestibular end organs were measured in normal temporal bones, but were limited to the superior as well as anterosuperior and posterosuperior margins of the footplate.

3. Fathoming of the vestibule below the central and inferior thirds of the footplate surface has shown that there is no likely danger for the vestibular end organs and cochlear duct if manipulations are carried out as deep as 1 mm below the surface.

4. The safest place for a stapedotomy opening is in the central and inferior-central third of the footplate.

5. The use of perforating instruments to achieve a stapedotomy opening is relatively safe over the entire surface of the footplate, if they are not introduced more than 0.5 mm into the vestibule.

6. A stapedotomy piston of 0.4 mm in diameter can be introduced relatively safely 0.5 mm into the vestibule over the entire surface of the stapedial footplate.

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ORTHOSTATIC POSTURAL CONTROL IN VESTIBULAR NEURITIS: A STABILOMETRIC ANALYSIS

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Posturographic data for 182 vestibular neuritis patients were recorded on a static posturographic platform (stabilometry) with the patients' eyes both open and closed. Three of the parameters studied are presented here: mean position of the center of pressure, area of the statokinesigram, and Romberg's quotient. Vestibular neuritis modifies the performance of the fine-postural system in unpredictable ways, varying with the subject. It may disturb either the mean position of the center of pressure or the integration of visual input. Sixty of the patients in this study did not use visual input to stabilize their position in relation to their surroundings. The value of stabilometry as a method for observing the integration of visual input in the control of orthostatic posture is discussed.

KEY WORDS — posturography, Romberg's test, stabilometry, vestibular neuritis, visual input.

INTRODUCTION

Studies in posturography[1] have progressively demonstrated the existence of a particular type of behavior concerned with regulating balance in orthostatic posture in humans.[2,3] This type of behavior, unlike other behavior regulating balance, is used when a person is standing immobile in his surroundings, to stabilize the body position within extremely fine limits. Thus this behavior has been called the fine-postural system (Gagey and Toupet, unpublished observations, 1988). It has some similarities to the fine-postural system in animals as described by Baron.[4]

The performance of the fine-postural system has been widely studied in normal humans by means of stabilometric platforms. The results are consistent, within known limits, both between different individuals and between different recordings for the same individual.[5,6] Therefore, alterations in this behavior in subjects with disease can now be studied, and we have done this in patients with vestibular neuritis.

MATERIALS AND METHODS

STABILOMETRY

Stabilometric recordings were made by using a computerized static posturographic platform conforming to the standards of the Japanese Society for Equilibrium Research,[7] in the standard conditions set forth by the Association Française de Posturologie,[5] in eyes-closed and eyes-open conditions. A visual target with a precise geometric structure was 90 cm away. Lighting was at 2,000 lux using filament light bulbs (50 Hz), with the subject's vision corrected if necessary.

From the data provided by the stabilometric signal, we analyzed

1. The average position of the center of foot pressure on a left-right axis, called the "X-mean," in accordance with the Kyoto conventions (Fig 1A).
2. The area of the 90% confidence ellipse, the ellipse containing 90% of the center-of-pressure positions sampled,[8] called the "area."
3. "Romberg's quotient" as proposed by Van Parys and Njiokiktjien,[9] which is the ratio of the "area" with the eyes closed to the "area" with the eyes open, multiplied by 100.

VESTIBULAR NEURITIS

One hundred eighty-two patients were selected for this study on the basis of diagnosis of vestibular neuritis established after clinical examination and functional evaluation (caloric testing, rotary pendular test, assessment of eye movement on a smooth-pursuit test, and examination of saccades and of the capacity to maintain extreme lateral gaze). The diagnostic criteria were as follows:

1. A single episode of intense rotatory vertigo lasting more than a few hours, with an absence of clinical auditory signs.
2. An acute unilateral deficit on caloric tests, of at least 50% (according to Jongkees' formula).
3. Spontaneous nystagmus contralateral to the deficit.
4. Absence of signs of central involvement.

This group was not homogeneous with respect to duration of the disease. In 39 cases the neuritis had lasted less than 3 weeks (mean, 12 days ± 6 days) and in 143 cases it had lasted longer. But the sex distribution was roughly even: 90 men and 92 women. Subjects of more than 60 years of age were excluded, as were patients suffering from clinically evident neurologic or orthopedic disorders.

STATISTICAL ANALYSIS

The theoretic normal distribution of posturographic parameters on a standardized platform, in

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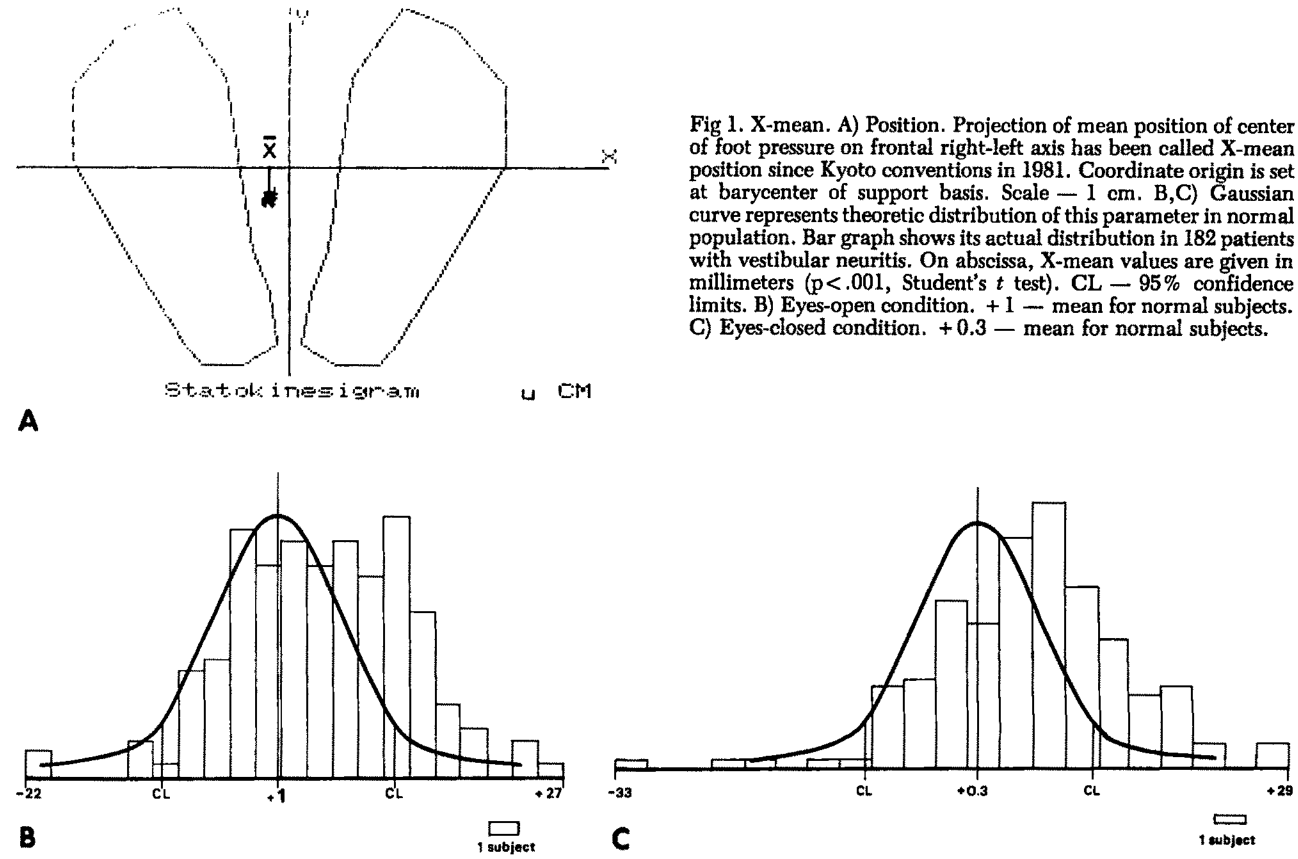

Fig 1. X-mean. A) Position. Projection of mean position of center of foot pressure on frontal right-left axis has been called X-mean position since Kyoto conventions in 1981. Coordinate origin is set at barycenter of support basis. Scale — 1 cm. B,C) Gaussian curve represents theoretic distribution of this parameter in normal population. Bar graph shows its actual distribution in 182 patients with vestibular neuritis. On abscissa, X-mean values are given in millimeters ($p<.001$, Student's t test). CL — 95% confidence limits. B) Eyes-open condition. + 1 — mean for normal subjects. C) Eyes-closed condition. + 0.3 — mean for normal subjects.

eyes-open and eyes-closed conditions,[5] was established from three series of measurements, each in a different cohort of healthy subjects (N [men + women]; age in years ± SD, age range).

1. 100 (50 + 50); 33 ± 10, 19 to 57 years.
2. 40 (40 + 0); 23 ± 4, 19 to 27 years.
3. 110 (55 + 55); 35 ± 11, 18 to 59 years.

Statistical tests used were Student's t test to compare the theoretic and observed distributions, the χ^2 test for contingency tables, and correlation coefficients.

RESULTS

SUBJECTS WITH VESTIBULAR NEURITIS

X-Mean. The distribution of the X-mean in the total population of neuritis patients was significantly different from the normal distribution ($p<.001$) both in the eyes-open (Fig 1B) and in the eyes-closed (Fig 1C) conditions.

However, only 20% of the neuritis patients had an X-mean outside normal limits, usually to the right side, without any other obvious cause. In comparison with the cohorts of normal subjects, the frequency of abnormal scores was significantly greater in both eyes-open and eyes-closed conditions (eyes-open: $\chi^2 = 14.3$; eyes-closed: $\chi^2 = 14.04$; 1 *df*; $p<.001$).

Area of 90% Confidence Ellipse. The distribution of the areas for the neuritis group with their eyes closed (Fig 2A) did not exactly overlap that for the normal subjects ($p<.05$), but only 29 patients had an area value above the upper 95% confidence limit.

The distribution of the areas for the patients with their eyes open (Fig 2B) was significantly different ($p<.001$) from that for the normal subjects, with 87 of the neuritis patients having an area value higher than the upper normal limit in this condition. The frequency of abnormal scores in the eyes-open condition was very significantly higher than that for the eyes-closed condition ($\chi^2 = 42.56$; 1 *df*; $p<.001$). Neither the age nor the visual acuity of this group of patients with abnormal area values was different from that of the whole population of patients.

Romberg's Quotient. The difference between the distribution of Romberg's quotient for neuritis patients and that for normal subjects was significant ($p<.001$; Fig 3). Sixty-three patients had a quotient below the normal limit, and all 63 had normal values for their parameter of area in the eyes-closed condition.

NEURITIS OF SHORTER AND LONGER DURATIONS

X-Mean. The distribution of the X-mean values in the patients with neuritis of recent onset (under 3 weeks) was not significantly different from that of patients with neuritis of longer duration (over 3

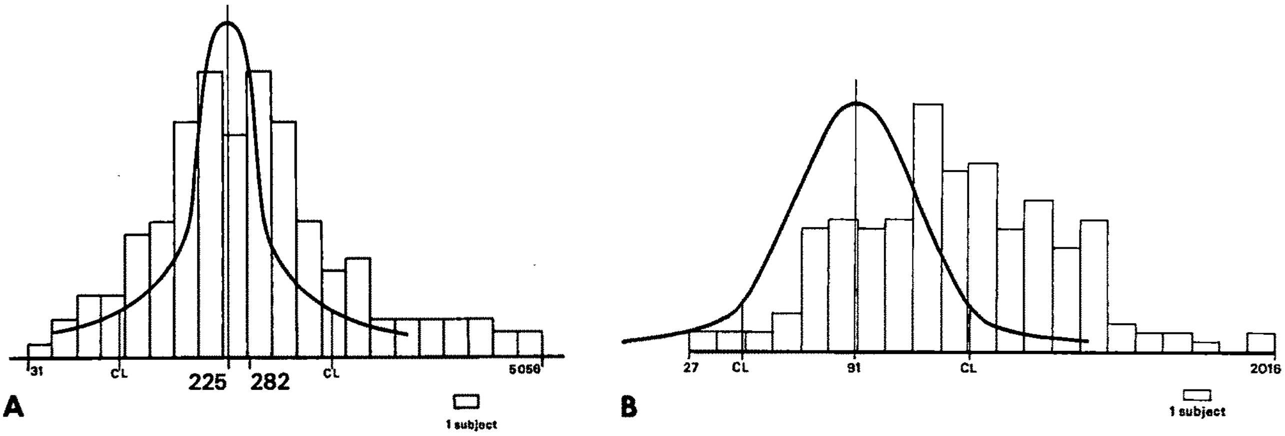

Fig 2. Area of 90% confidence ellipse. Gaussian curve represents theoretic distribution of this parameter in normal population. Bar graph represents its actual distribution in 182 patients with vestibular neuritis. On abscissa, area values are given in square millimeters. Scale is logarithmic. CL — 95% confidence limits. A) Eyes-closed condition ($p < .05$, Student's *t* test). 225 — mean for normal subjects, 282 — mean for vestibular neuritis. B) Eyes-open condition ($p < .001$, Student's *t* test).

weeks), in either the eyes-open or the eyes-closed condition. The frequency of abnormal scores was not significantly different in the two cohorts ($\chi^2 = 1.8$; 1 *df*).

Area. The distribution of the area values for the patients with recent neuritis was significantly different from that for patients with longer-term neuritis (eyes open, $p < .02$; eyes closed, $p < .01$). After 3 weeks of the illness, the areas had already decreased.

Romberg's Quotient. The distribution of Romberg's quotient did not differ significantly according to the duration of the illness. The frequency of abnormal Romberg's quotients was the same in the two cohorts ($\chi^2 = 0.15$); illness lasting more than 3 weeks did not improve the use of visual cues, at least over the observed period.

ABNORMAL X-MEAN AND ABNORMAL ROMBERG'S QUOTIENT

The contingency table for neuritis patients with normal versus abnormal X-means and Romberg's quotients (see Table) shows that these ill effects of neuritis are independent ($\chi^2 = 1.58$; 2 *df*). Moreover, the correlation between these two parameters was very low, both in the neuritis patients ($r = -.09$) and in the normal subjects ($r = .10$).

DISCUSSION

When standing upright with their eyes closed, most of the patients with vestibular neuritis were as steady as the normal subjects; it was only when the patients opened their eyes that some of them became unsteady. No consistent, systematic alteration of any of the posturographic parameters was found. Vestibular neuritis affected the fine-postural system in an erratic way, varying with the subject. But what does that mean?

Norré and Forrez[10] think that stabilometry leads to better understanding of vestibular patients and to better adaptation of their treatment. Actually, we found two distinct subgroups of vestibular neuritis patients: those with an abnormal X-mean and those with an abnormal Romberg's quotient.

In our experience an abnormal X-mean, in the absence of any orthopedic disorder, was a sign of abnormal asymmetry of postural tone. This tonic postural asymmetry is well known in vestibular neuritis patients, and the results of this preliminary study merely show that it could be useful to monitor such asymmetry in vestibular neuritis more closely by using posturographic recordings; as far as we know, no study of this particular type of follow-up has been published.

As this study of Romberg's quotient in vestibular neuritis seems to be the first,[10-12] we shall expand on the significance of the surprising fact that as regards posture, some patients behaved as if they were blind: they did not use vision to keep steady, and so they could be called "posturally blind" — a dangerous term, but convenient for summing up the observed phenomenon.

Stabilometry appeared very early to be the method of choice for quantifying Romberg's test.[13] But the pioneers of posturography did not attempt to distinguish among the various effects of opening the eyes such as visual control over postural sway, an activation reaction of the nervous system structures regulating posture.[14] In the absence of basic research, the "eyes-open" phenomenon has been considered in the aggregate. The recording conditions for subjects "with eyes open" have varied considerably from one laboratory to another. Subjects have been placed in semidarkness, for example, with a single visual target: a fluorescent bar 5 m away.[15] It was not until 1978 that Bles et al[16] showed how visual stabilization depended on the distance from the visual target. Paulus et al[17] then systematically studied the parameters of visual input in the fine-postural system: distance from the

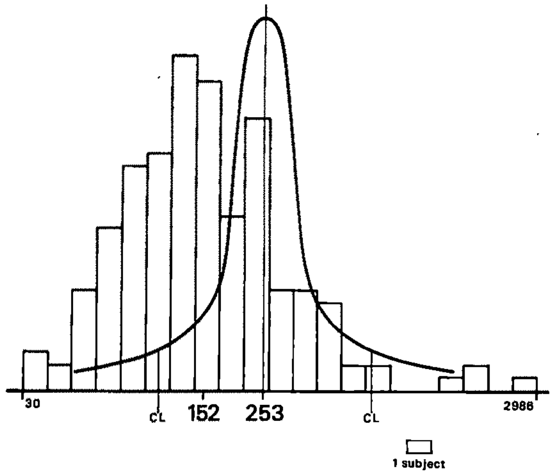

Fig 3. Romberg's quotient. Gaussian curve represents theoretic distribution of this parameter in normal population. Bar graph shows its actual distribution in 182 patients with vestibular neuritis. On abscissa are values of Romberg's quotient ($p < .001$, Student's *t* test). Scale is logarithmic. 253 — mean for normal subjects, 152 — mean for patients with vestibular neuritis, CL — 95% confidence limits.

visual target, visual acuity, frequency of the lighting, geometry of the visual target, and involvement of the central and the peripheral retina. Today we can no longer study Romberg's quotient in stabilometry without referring to that work and standardizing the visual conditions for recording in the eyes-open condition.[5,18]

For normal subjects tested in these standardized conditions, the areas we found for the eyes-closed condition were always larger than those for the eyes-open condition — indeed, very much larger: 250% on average.[5,17] Therefore, whatever its cause, the behavior indicating postural blindness under our recording conditions is certainly abnormal statistically, although what it signifies is less clear.

It does not seem to be necessarily a sign of a lack of visual information available to the nervous structures that participate in the control of posture. In another study it was noticed that in some subjects one can provoke the immediate appearance of such postural blindness merely by disturbing their oculomotor system (for instance by supplying badly centered corrective lenses); when this disturbance is eliminated the postural blindness disappears immediately.[19] Thus, visual information may be available but ineffective. We suggest that the phenomenon of postural blindness indicates that visual input is being abnormally integrated into the multimodal system controlling orthostatic posture.

According to the logic of this system, variations of body position relative to the environment can be perceived only by sensory organs in relation with this environment: retina, utricular macula, saccular macula, cupula, and mechanoreceptors of the sole of the foot. As these "exo-inputs" are located in parts of the body that are movable in relation to each other, integrating the positional information they provide requires complementary information on their relative position, and that is the role of proprioception. Furthermore, logically the retinal signal cannot be used in isolation by this system, because the postural significance of retinal slip is ambiguous: such a slip may be due to movement of the body, or of the surroundings, or of the eyes. Therefore, the retinal signal must be compared with signals from other inputs before being used by the fine-postural system.

CONTINGENCY TABLE FOR NEURITIS PATIENTS

	Abnormal X	*Normal X*	
Abnormal RQ	17	46	63
Normal RQ	43	76	119
	60	122	182

RQ — Romberg's quotient, X — position of X-mean.

Experimentally, it is precisely when this essential stage of integration of visual input is disturbed by failure of another input that we observe postural blindness: when there is a disorder of the vestibular input, as in this study, but also when there is a disorder of the oculomotor input, as in heterophoria or heterotropia,[20] or a disorder of the spinal proprioceptive input, as in acute lumbar pain (R. Gentaz, unpublished observations). It is as if the visual input were no longer reaching its threshold of effectiveness for lack of another input.

It was already known, and this study confirms, that even without vision the fine-postural system can maintain the position of the center of gravity within very narrow limits, but a lack of integration of visual input into the control of orthostatic posture appears only when Romberg's quotient is studied.

When the center of gravity shifts to outside the narrow limits controlled by the fine-postural system (Gagey and Toupet, unpublished observations, 1988), the functional situation is different: balance is threatened.[21,22] Several studies made in such destabilizing conditions have underlined the importance of vision in vestibular compensation, both in animals[23] and in humans.[24,25] The functional organization of the central nervous system adapts to different conditions, and is different in static and dynamic situations.

CONCLUSIONS

Posturography is of no interest for the diagnosis of vestibular neuritis.[24] Practitioners and clinicians knew how to establish this diagnosis long before stabilometric platforms were ever built. The interest of a stabilometric evaluation in vestibular neuritis lies in showing how all the patients whom we lump into a single nosologic category in fact have widely different behaviors for regulating and controlling their orthostatic posture. To treat such patients it is no longer enough to verify that they see clearly, that the soles of their feet perceive pressure, or that their proprioception is normal. The analysis must be taken further to answer the question of whether or not the available postural information is being integrated by the fine-postural system. A postural checkup should be able to help answer this question.

Finally, this study of clinical stabilometry confirms earlier work[26] on the unique, specific nature of the behavior regulating orthostatic posture in humans.

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TULLIO PHENOMENON AND POSTURAL STABILITY: EXPERIMENTAL STUDY IN NORMAL SUBJECTS AND PATIENTS WITH VERTIGO

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The effect of low-frequency sound (LFS) on postural stability was studied in 55 healthy volunteers and in 152 patients with vertigo due to different types of inner ear disease. The sound pressure levels ranged from 130 to 132 dB and were given at frequencies of 25, 50, and 63 Hz. The duration of each stimulation lasted 30 seconds. The postural responses were measured with a force platform. The LFS stimulation improved the postural stability of the healthy subjects through the alerting response. We did not observe any difference in the body sway according to whether the LFS was delivered with monaural or binaural stimulation. Twenty-six percent of the patients with Meniere's disease, 40% with chronic otitis media with vertigo, and 26% with vertigo of peripheral origin experienced significant postural instability at least at two of the three test frequencies during stimulation with LFS. The results demonstrate that in subjects with different types of inner ear disease the vestibular system starts to respond to LFS. The activation of vestibulospinal responses by LFS seems to be an expression of the Tullio phenomenon.

KEY WORDS — chronic otitis media, Meniere's disease, perilymph leak, posturography, Tullio phenomenon, vertigo.

INTRODUCTION

It has been assumed that low-frequency noise may affect the vestibular end organs directly.[1,2] Noise in an audible frequency range can cause vertigo when it occurs suddenly[3,4] or changes in character.[5] The sudden dizziness that sometimes occurs in aerospace, or that industrial workers experience, has been explained either as a direct effect of sound on the labyrinth or as a central arousal reaction of the brain stem.[4,6] In response to sudden sound stimulation, nystagmic eye movements can be observed. These represent a sound-induced vestibular stimulation[7,8] known as the Tullio phenomenon.

Tullio[1] originally showed that sound-induced nystagmus arises by transmission of sound energy in the inner ear fluids. He made microscopic holes in the horizontal semicircular canal and used the dye particle technique to show that sound waves spread from perilymph to endolymph and stimulate the hair cells of the crista ampullae. In the Tullio phenomenon sound pressure induces electrical potentials equivalent to cochlear microphonics from different receptors of the vestibular labyrinth.[2] Originally, it was assumed that only a fistula in the bony capsule could be the cause of sound-induced vertigo.[8] Furthermore, the Tullio phenomenon was considered to be pathognomonic for bone softening in the luetic inner ear.

The Tullio phenomenon has been reported also in patients with Meniere's disease,[9-11] and also as a result of chronic middle ear infection when abnormal adhesions between the stapes footplate and the inner ear have developed.[12] Some authors have even regarded the Tullio phenomenon as a normal sign during high sound pressure levels (SPLs), but because of methodologic faults the data are not conclusive.[13]

So far the prevalence of the Tullio phenomenon has not been systematically explored in patients with vestibular dysfunction. Moreover, its specificity with regard to type of lesion is not well known. The purpose of the present work was to evaluate the effect of low-frequency sound (LFS) stimulation on postural stability in normal subjects and in patients with vertigo due to different types of inner ear disease with vestibular problems.

MATERIAL AND METHODS

Subjects. The normal values consisted of recordings made in 55 healthy volunteers. The subjects were exposed to LFS in both ears simultaneously and in a separate session in one ear only. The median age of the subjects was 35.0 years (range, 18 to 55 years). They were all working at the Department of Otolaryngology, University Hospital of Helsinki. In addition, 152 patients with different otologic diseases were examined. These patients were selected from a larger group of patients visiting the ambulatory unit during the years 1988 and 1989. The experimental protocol was reviewed and approved by the Ethics Committee of the University of Helsinki as regards human experimentation.

Fifty of the patients suffered from Meniere's disease (median age, 47.0 years; range, 22 to 77 years).

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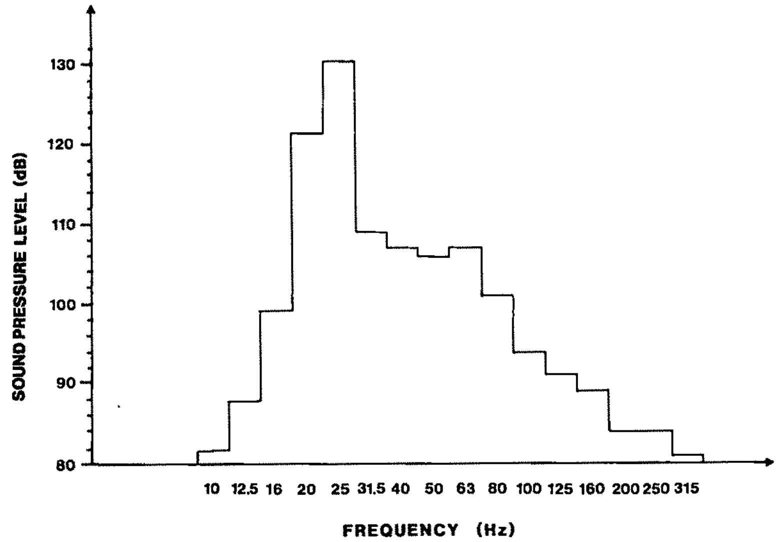

Fig 1. Frequency distribution in one-third-octave bands for 25-Hz sound stimulation signal.

Twenty-five patients had chronic otitis media (COM) with vertigo (median age, 54.0 years; range, 32 to 79 years). Most of these patients had been subjected to previous operations on the middle ear. In five of them a cholesteatomatous fistula in the horizontal semicircular canal was suspected and later confirmed. Seventy-seven patients suffered from vertigo that was linked to peripheral vestibular malfunction (median age, 45.0 years; range, 20 to 72 years). This patient group included those with vestibular neuronitis and nondefined vestibulopathy of peripheral origin with recurrent attacks of vertigo with or without concomitant hearing loss. In 8 of them an exploratory tympanotomy was performed to reveal a perilymphatic fistula (PLF).

Equipment. The measuring system comprised a computer-controlled stimulator and a posturography unit.[14-16] A force platform technique was used. The force platform is constructed with the strain gauge principle, and the system evaluates changes in the center point of force on the platform surface. The signal is fed into a computer, and the analysis evaluates the relative displacement of body position during quiet stance and during perturbation of the posture with LFS. A desktop computer (Hewlett-Packard 9000, series 300) was used in sampling and analyzing the data. For baseline measurement and each stimulation frequency, the parameters were calculated, printed on paper, and stored on disk for further statistical analysis.

The LFS was generated with a piston-type air sampling pump (portable pump model S, Mine Safety Appliances Co, Pittsburgh, Penn) that has an adjustable stroke frequency ranging from 5 to 70 Hz. The ears of the subjects were covered with tight earmuffs (Silenta Ergo, Kemira, Finland) connected with a hard silicone tube (diameter, 5 mm) to the pump. The test sequences were controlled with a microcomputer (Hewlett-Packard 75C) through a programmable power supply (Hewlett-Packard 50501A and 6200B).

The sound level and frequency were measured with an artificial head (ISO/DIS 6290) and a one-

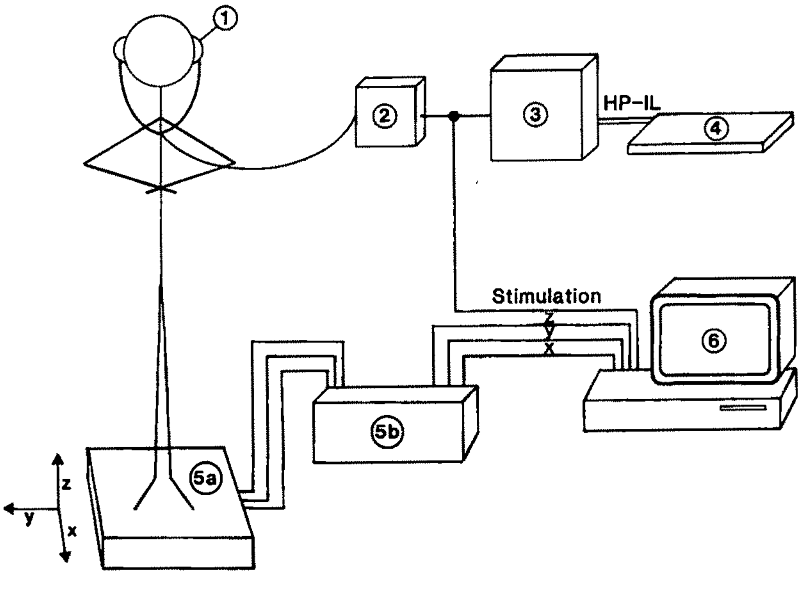

Fig 2. Block diagram of equipment used in test. 1 — earmuffs, 2 — piston-type air pump, 3 — computer-controlled power supply, 4 — hand-held microcomputer, 5a — force platform, 5b — amplifiers, 6 — microcomputer with analog-to-digital converter, HP-IL — Hewlett-Packard interface loop, x — fore and aft, y — lateral, z — vertical.

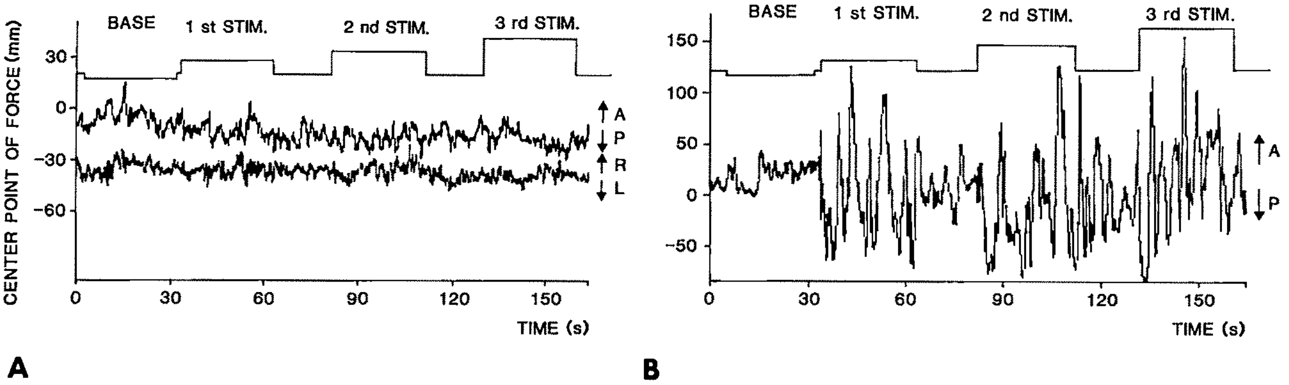

Fig 3. Effect of low-frequency sound on posture. Ordinate indicates position of center point of force in millimeters. Abscissa indicates time in seconds. A) Normal subject. Uppermost trace indicates order and timing of baseline and stimulation periods. Middle trace shows center point of force in anteroposterior direction, and lowest trace, in lateral direction. B) Patient with vertigo. Stimulation periods and anteroposterior position of center point of force are shown.

third-octave band real-time analyzer (Brüel & Kjaer 2131). The shape of the signal was almost sinusoidal. The maximum amplitude of the stimulation signal occurred at the indicated one-third-octave band frequency, and the neighboring one-third-octave bands were at least at a 10-dB lower level than the indicated one (Fig 1). The stimulation frequencies in the test were 25, 50, and 63 Hz, at a constant SPL of 130, 132, and 132 dB, respectively.

In addition to the LFS, the pump caused a negative pressure that when measured with an artificial head was 80, 315, and 495 mm H_2O at the frequencies tested. When measured with the human head the negative pressure values were 66, 150, and 250 mm H_2O at the frequencies tested (mean of five subjects). This lowering of the values was due to leakage of air between head and earmuffs. The LFS was superimposed on top of the negative pressure. A block diagram of the test equipment is shown in Fig 2.

Test Sequence and Parameters Used in Analysis. The subjects stood during the measurements with their eyes closed, heels together, arms crossed over the chest, and feet at 30° angles. The quiet stance was recorded after initial sway stabilization during a 30-second period immediately before the first stimulation period. Three stimulation sequences were arranged in ascending order to provide greatest stimulus intensity at the end of the test. Each stimulus period lasted for 30 seconds followed by a 20-second nonstimulation period. The sequences were the same for all subjects. Each stimulus frequency was delivered once to each subject.

In the present study sway velocity was used to determine the degree of postural stability. This parameter is calculated from the movement of the center point of force in anteroposterior and lateral directions (length divided by time). It was calculated during baseline stance (nonstimulated condition) and during the different stimulation periods. Sway velocity during the baseline period was used as a reference value to indicate undisturbed postural control activity. Sway velocity values at different LFS frequencies were compared to the baseline value as a measure of the effect of LFS.

Statistics. The differences between the healthy controls and patients were tested by one-way analysis of variance (ANOVA). In pairwise comparison between the patients and controls and between unilateral and bilateral stimulation, the significance of the differences was tested with Student's *t* test. The calculated p values of the different stimulus conditions were then used in a second-stage analysis of summary statistics with the formula

$$T(m) = -2 \sum_{i=1}^{m} \log P i$$

in which i is the number of the test, P the significance value of the test, and m the number of the last test and thus the total number of tests. T(m) will correspond exactly to the χ^2 distribution with 2m *df*.[17]

RESULTS

Individual Recordings. Figure 3A gives an example of an individual recording made from a normal subject during bilateral stimulation and Fig 3B gives an example from a subject with an inner ear disease. The sway path traces in Fig 3A are separately given for the anteroposterior and the lateral directions. In Fig 3B, only the anteroposterior direction is shown to illustrate the postural instability in this direction. When comparing Fig 3A with Fig 3B, one should note that the ordinate is given in a different scale. The normal subject (Fig 3A) displayed unchanged body sway during baseline and stimulation periods. The patient (Fig 3B) had almost normal body sway during the baseline recording, but stimulation induced a powerful ataxia in postural stability. The responses of this patient did not depend on frequency, and already at the lowest stimulation frequency the body sway was significantly increased. This subject belonged to a group

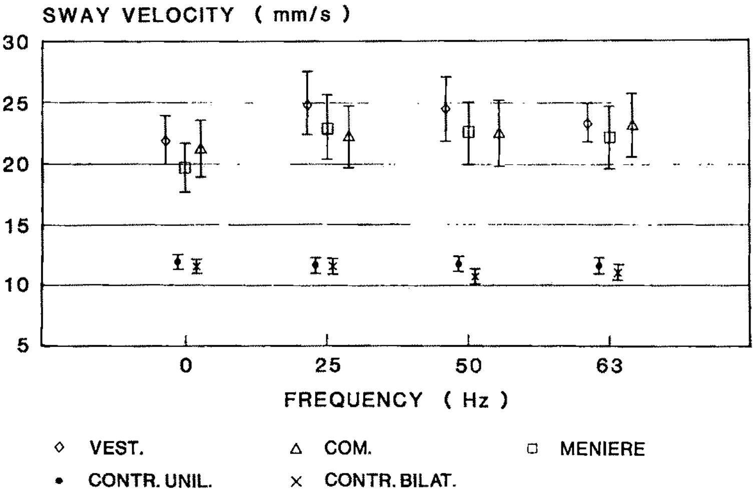

Fig 4. Average body sway velocities at different stimulation conditions in different groups of patients and in control subjects. Means and SEM are shown. Vest — peripheral vestibular dysfunction, COM — chronic otitis media with vertigo, contr unil — control unilateral stimulation, contr bilat — control bilateral stimulation.

of patients with vertigo of peripheral origin, and in exploratory tympanotomy in the ear with symptoms, we found an incomplete fusion line (fissura) running from the oval window to the round window. Covering the fissura with fascia and tissue glue improved the vertigo and removed the LFS sensitivity. After 6 months the patient started to suffer again from vertigo and the LFS stimulation yielded pathologic results. A renewed tympanotomy and fascia coverage above the fissura area cured his symptoms and changed the LFS test results to normal. The repeated LFS tests conducted 3 and 6 months later showed normal results.

Average Responses. The healthy control subjects showed slight improvement of their postural stability during LFS stimulation (Fig 4). The difference between stimulation condition and baseline stance was statistically significant ($\chi^2[8] = 21.69$, $p < .001$). In pairwise comparisons (Student's *t* test) we found significant differences between baseline conditions and the 50-Hz stimulation period ($t[54] = 3.99$, $p < .001$) as well as the 63-Hz stimulation period ($t[54] = 1.92$, $p < .05$). We did not observe any differences between unilateral and bilateral sound stimulation ($\chi^2[8] = 13.37$, p not significant).

The mean responses for the patients with Meniere's disease, those with COM and vertigo, and those with vertigo of peripheral origin are shown in Fig 4. The sway velocity even during baseline recording was significantly increased in all groups of patients (ANOVA, $p < .001$) when compared with the control subjects. Stimulation with LFS further increased the sway velocity in the patients, and the difference between control subjects and different groups of patients was statistically significant (ANOVA, $p < .001$).

The relative increase in sway velocity was inspected by studying the quotient between sway velocity recorded during the stimulation period and that recorded during the baseline period for each group of subjects (see Table). In all stimulation frequencies the patients showed significantly higher quotient values than did the controls (ANOVA, $p < .05$). At the lowest stimulation frequencies the velocity quotient was increased in patients with Meniere's disease by a factor of 1.2, in COM with vertigo by 1.08, and in patients with vertigo of peripheral origin by 1.18. When compared pairwise with the controls (Student's *t* test), the responses differed statistically from those of the control subjects in patients with Meniere's disease ($t[103] = 2.65$, $p < .01$) and in the patients with vertigo of peripheral origin ($t[130] = 1.96$, $p < .05$). At 50-Hz and 63-Hz stimulation in pairwise comparison, all groups differed highly significantly from the controls ($p < .001$).

Individual Pathologic Responses in Different Groups. The number of subjects exceeding the 95% range of normal values (measured as relative increase) during at least two stimulation frequencies is given in Fig 5. A high number of patients in different disease groups responded with increased sensitivity to LFS. Thus, 13 of 50 patients with Meniere's disease, 10 of 25 patients with COM with vertigo, and 20 of 77 patients with vertigo responded with increased body sway during exposure to LFS.

DISCUSSION

The present study focused on the postural stability of normal subjects and patients with vestibular and/or cochlear lesions when exposed to LFS. The postural responses were analyzed on the force platform by recording the movement of the center point

QUOTIENTS DESCRIBING RATIO BETWEEN STANCE DURING STIMULATION PERIOD AND THAT DURING BASELINE CONDITIONS (MEAN±SD)

Stimulus	*Control (n = 55)*	*Meniere's Disease (n = 50)*	*COM (n = 25)*	*Vertigo (n = 77)*
25 Hz	1.00 ±0.18	1.20 ±0.51	1.08 ±0.33	1.18 ±0.66
50 Hz	0.93 ±0.16	1.14 ±0.45	1.12 ±0.42	1.20 ±0.70
63 Hz	0.95 ±0.19	1.13 ±0.45	1.17 ±0.46	1.17 ±0.65

COM — chronic otitis media.

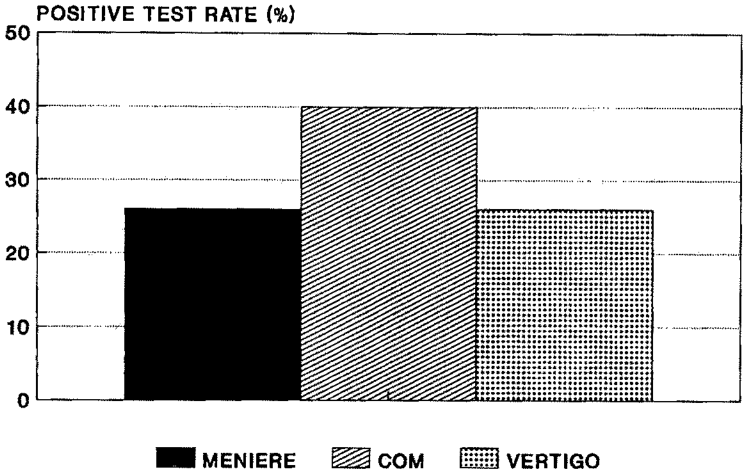

Fig 5. Prevalence of pathologic responses in low-frequency sound test in different groups of patients. Vertigo — peripheral vestibular dysfunction, COM — chronic otitis media with vertigo.

of force.[14-16,18] Other possible parameters used are the length of the body sway path during the nonstandardized measurement period, the sway area, and the frequency content of the body sway. We chose sway velocity, which evaluates the body sway path divided by time. Thus, it is not time-dependent, but it evaluates the length of the body sway. The sway velocity easily detects changes in corrective movements of posture (occurring at high frequencies), whereas it is not very effective in detecting slow position shift in posture.[19] Nevertheless, the slow body shifts can be easily observed from the chart. In only one patient a slow consistent deviation of the body posture was observed that was consistent with repeated recordings. In most subjects the LFS caused rapid body posture shifts that could be recorded better as velocity change during stimulation than as a deviation of body position.

In the present experiments the SPL ranged from 130 to 132 dB when measured with a standardized artificial head. This head has flat metal surfaces that make a perfectly airtight fit between the head and the earmuffs, which cannot be obtained with human heads with their wide variety of forms and hairstyles. Additional measurements with the artificial head indicated that leakage through a 3-mm hole between the cushion ring and the artificial head reduced the amplitude of the peak frequency by as much as 30 dB. It should be noted that in the case of leakage the first and second harmonics of the stimulation sound remain at the original level. This means that the peak frequencies shift upward with decreased amplitude and subjects with poor fit may be stimulated by a lower SPL and higher peak frequency than subjects with a good fit of earmuffs. This may expose the patients with COM with vertigo to higher frequencies of LFS than other subjects, since after a temporal bone operation the mastoid area will be somewhat deformed and the possibility of a leak will be increased.

Most of the research dealing with acoustic, vestibular, or LFS stimulation has used a unilateral stimulus presentation paradigm.[20] A unilateral stimulus is usually chosen because in a normal subject a binaural stimulus may cancel the effect at the level of the brain stem (unless there is an abnormal condition such as vestibular or cochlear disease). In the present work we could not demonstrate that in the normal subjects a unilateral stimulation would differ from a bilateral stimulation by causing postural instability. We preferred to use a bilateral stimulus since both ears have to be screened at the same time. Moreover, in natural conditions the LFS stimulation is binaural and only the shadow of the head influences the strength of the perceived stimulus.

In normal subjects the LFS did not produce an increase in body sway with either unilateral or bilateral stimulation. Moreover, the normal subjects showed a tendency to improve their postural stability during stimulation, probably through the alerting response generated by the LFS.[3,6] Nevertheless, in several patients the LFS provoked postural instability that in many cases caused a risk of a fall. This indicates that subjects with vertigo are not good candidates for work tasks in which they are exposed to a high SPL of noise. The LFS levels in the present study were high but still less than the permissible SPL at low frequencies in Finland. When looking at the current ratings from a sensitive patient's point of view, the present norms are not adequate. Presumably some screening and exclusion should be used in the selection of workers who have to be exposed to LFS. It is noteworthy that in most work places with high levels of LFS, whole body vibration is also found.[21] Because whole body vibration also induces postural instability,[18,22] the combination with LFS may cause cumulative difficulties in postural stability.[21]

It is still unsettled whether LFS at an intensity of 130 dB can cause a hearing loss. In normal subjects Slarve and Johnson[23] did not observe changes in the permanent hearing threshold after exposing the subjects to LFS at frequencies of 12.5 to 30 Hz at SPLs ranging from 125 to 140 dB. These results were later confirmed by Mohr et al.[24] Nixon and Johnson[25] and Nixon[26] exposed healthy volunteers to

LFS at 135 to 140 dB SPL for 5 minutes or more and observed that in some of the subjects a temporary threshold shift of 15 to 25 dB was observed. The temporary threshold shift subsided within 30 minutes. The present results demonstrate that LFS exposure differs in subjects with an inner ear lesion and in normals. The safety limits for normals may therefore not be accurate, as mentioned above. The subjects with an inner ear disorder may be more vulnerable to LFS than normals, as their postural responses also indicate. Greater attention should be paid to these subjects until the safety limits for these subjects have been determined.

Even a healthy vestibular end organ is capable of detecting sound at a high SPL.[2,27] The threshold sensitivity varies between the semicircular canals and the otolith organ. In the monkey ear the saccule afferents respond to sound at an SPL of 106 to 110 dB, and the respective value for semicircular canal afferents is 120 dB SPL. These responses are phase-locked with nerve potentials at frequencies up to 250 to 350 Hz. When the frequency-dependent sensitivity of the vestibular system to LFS is taken into consideration, it is evident that in the present experiment the saccule afferents of a healthy subject would respond to LFS at an SPL exceeding 145 dB. We did not try to reach such a high threshold level in the present experiment because of the annoyance such an SPL produces in the ear. Confirming this idea, Parker et al[27] reported (in testing somewhat higher frequencies than we did) that vestibular responses may occur at an SPL only exceeding 138 dB.

The vestibulospinal responses during LFS stimulation in the present study seem to be similar to the vestibular responses found in the Tullio phenomenon.[1,7] Nevertheless, in the present stimulation mode the pump also generated a negative pressure in the ear canal. Creating an underpressure with simultaneous LFS loading (pulling out of the stapes and improving its mobility) has been considered to improve the sensitivity of the Tullio phenomenon. In this respect the LFS stimulation also generated test conditions that may provoke Hennebert's sign.[28] Hennebert observed that an underpressure or overpressure in the ear canal is able to cause nystagmus and instability in patients who have luetic softening of the bony capsule.[28] Later it was concluded that also patients with PLF often show a positive Hennebert's sign, and the test is advocated for detecting a "third window" in the labyrinth.[11,29]

The classic "third window syndrome" may signify a PLF,[29] intracochlear adhesions,[12] or rupture of membranes limiting the endolymphatic space.[8,30] Since in the present study some of the patients with the positive LFS response might have suffered from a PLF,[30-32] we performed tympanotomy in eight patients with clinical symptoms mimicking PLF. We could not confirm visible leaks in the round or oval window, although covering the windows with fascia led to disappearance of LFS sensitivity in four patients. In one patient we observed a fissura in the promontorium. A covering of this fissura with fascia led to the disappearance of the Tullio phenomenon. Thus, in four of the patients with a potent response to LFS, a PLF may exist. In these four patients, a clinically positive Hennebert's sign correlated well with the LFS test result. We are aware that Black et al[29] indicated high selectivity and sensitivity with their LFS test conducted on posturography for detection of PLF. So far, on the basis of our experience, it is not possible to establish any criteria for detection of a PLF with the LFS loading test alone because of its nonspecificity and high number of false-positive reactions in our hands. There are, however, some basic differences between the test used by Black et al[29] and the one we used, especially concerning the frequency range of the stimulus, that may explain the differences in the results of the test.

The high rate of a positive Tullio phenomenon in patients with COM with vertigo may be explained by the nature of their disease and selection of the patients. They all have had a long-standing suppurative or cholesteatomatous process in the middle ear that has had an impact on hearing by production of a sensorineural component in their hearing loss. In addition, they all suffered from vertigo indicating that they also had vestibular involvement. It is a common observation that many patients with ears successfully operated on for COM and clinically healed will develop vertigo later in their life but often do not show any sign of a recurrence of previous middle ear disease. It is possible that some kind of "fibrosis" may develop in the vestibulum that later may cause disturbances by changing the stimulus selectivity of the vestibular system, as also occurs in Meniere's disease. Thus, instead of responding to head position and acceleration below 6 to 10 Hz, the vestibular system of these patients starts to respond to stimulation at higher frequencies.

In five patients with COM with vertigo we observed a cholesteatomatous fistula in the horizontal semicircular canal. Two of them had a positive Tullio phenomenon. Nevertheless, in the three other patients with COM and vertigo the test was negative despite an operative finding of cholesteatomatous fistula. Thus, the LFS test is not specifically effective in revealing a cholesteatomatous fistula. In these patients we saw a disparity between Hennebert's sign and LFS test results. Hennebert's sign was easily provoked in all these patients. A possible explanation is that the massive amount of tissue covering the fistula could not be brought into vibration with the current frequency content of LFS. In the near future we will start to investigate the effect of lower frequencies (at infrasound range) on the posture of patients with vertigo.

The reason that a significant number of patients with Meniere's disease have pathologic postural responses to LFS is not understood. It may be an expression of the well-known paracusis phenomenon, in which loud sound in these patients produces pain and dizziness. A careful history of unsteadiness sensation could confirm this in some of the responders. It can also be speculated that during large stapes movements, the stapes footplate may hit the saccule and/or utricle and mechanically stimulate the otolith system. Such a mechanism may not be very likely, since in the present experiments the underpressure kept the stapes at a distant (lateral) position. Still another possible explanation is that scarring and adhesions from the footplate to the membranous labyrinth may arise during rupture of the membranous compartments in attacks of Meniere's disease.

CONCLUSIONS

In the present study we exposed healthy volunteers and patients with vertigo of peripheral origin to LFS at a level of 130 to 132 dB. In the control subjects the LFS tended to improve the postural stability. The results were essentially the same whether monaural or binaural stimulation was used. The patients with vertigo of a different origin often responded to LFS with increased postural instability. We interpret the LFS responses as a sign of the Tullio phenomenon, in which sound stimulates the vestibular part of the inner ear. In a very limited number of cases a definite disorder, such as a PLF, enlarged fusion lines of the bony labyrinth, or fibrous adhesions between the stapes footplate and saccule, may exist. In the majority of the patients no definite cause for LFS sensitivity could be shown. Thus, a nonspecific vestibulocochlear lesion is able to change the dynamic frequency range of the inner ear receptor systems. Although the LFS levels in the work place seldom reach the levels used in this study, patients with vestibular disease may have an increased risk of postural instability in work places with high levels of LFS.

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NINTH SHAMBAUGH/SHEA WEEKEND OF OTOLOGY

The Ninth Shambaugh/Shea Weekend of Otology will be held March 6-8, 1992, at the Chicago Marriott Downtown in Chicago, Illinois. For further information, contact John J. Shea, MD, 6133 Poplar Pike, Memphis, TN 38119; Phone: (901) 761-9720; Fax: (901) 683-8440.

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TWENTY-SEVENTH POSTGRADUATE COURSE IN EAR SURGERY

The 27th Postgraduate Course in Ear Surgery will be held from April 26 to May 1, 1992, in Nijmegen, the Netherlands. For further information and registration form, please write to Prof Dr P. van den Broek, University Hospital Nijmegen, Department of Otorhinolaryngology, PO Box 9101, 6500 HB Nijmegen, the Netherlands; Fax: 80-540251.

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INEXPENSIVE COCHLEAR IMPLANT DEVICE

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We have developed a cochlear implant (CI) device modified from the House/3M cochlear implant device. The cost of raw materials was about $25. We used a new and simple technique for coating the implanted coil. We modified the circuit and removed the amplitude-modulated circuit. With this modification, the device uses less electricity and fewer transistors. There are slightly more than 3,000 patients using CI devices all over the world. Millions of profoundly deaf patients are poor and cannot afford the CI device that is now commercially available. Any university with well-trained otolaryngologists and physicists or electrical engineers can perform this technique.

KEY WORDS — cochlear implant.

INTRODUCTION

William F. House first implanted a cochlear implant (CI) device in a patient in 1972; it is essentially this same device that is in use today.[1] There are slightly more than 3,000 patients worldwide using these CI devices (C. Dobson, Cochlear Corp, personal communication, 1989). The cost of most CI devices is more than $5,000. The House/3M CI device was considered to be simple and relatively inexpensive and had worldwide established service centers,[2] but many profoundly deaf patients are poor and cannot afford it. To solve this problem, we tried to make an inexpensive one, and with Dr House's help we modified the circuit from the House/3M CI device.[1,3,4] We made a CI device from raw materials costing $25. We used a new technique for coating the implanted coil. This made the overall expenses low. We modified the circuit and coils and removed the amplitude-modulated circuit. With these modifications, the device uses less electricity and fewer transistors. To our knowledge nobody else has made such a device.[1]

MATERIALS AND METHODS

This CI device has been modified from the House/3M CI device.[1,3] It consists of an internal coil, an external coil, and an external stimulator.

Internal Coil. The electrodes are pure platinum wire with a diameter of 0.2 mm and a 0.5-mm ball at the end. The active lead is 78 mm in length from the edge of the coil body to the ball end, with 6 mm left uninsulated to form the electrode. The ground lead is 68 mm in length, with 50 mm left uninsulated to form the electrode.

The implanted coil is wound on a ferrite pot core (15 mm in diameter and 4 mm thick) with 1,800 turns of 45-gauge copper wire. The platinum electrode leads are connected to the coil wires by way of a solder joint, and the coil windings are impregnated with cyanoacrylate glue to fill the voids between the coil turns. The coil and the platinum wires are then insulated with Selleys Super Steel Epoxy Filler/Adhesive (from Selleys Chemical Company, Bankstown, Australia) to increase long-term reliability, as it is water-resistant and a good insulator.

A thin layer of Silastic medical adhesive silicone type A (SMA) is used to coat the outside of the insulated coil and the platinum wires. Reinforced Silastic sheeting (SSR) is used to wrap the coated coil. It adheres to the coated coil through use of SMA. The coated platinum wires are insulated with SMA and silicone rubber tubing (inside diameter, 1.1176 mm; outside diameter, 2.159 mm). A 6-0 nylon suture is used to secure the edges of the SSR to the other SSR, and silicone rubber tubing to the SSR. The CI device is gas-sterilized, and then all edges and the ends of the silicone rubber tubing are sealed with SMA. The SSR is 0.1778 mm thick, and the implanted coil is 23 mm in diameter and 6 mm thick. The electrical properties of the coil exhibit a DC resistance of about 500 Ω.

External Coil. This is made from 1,500 turns of 42-gauge copper wire wound on a ferrite pot core (15 mm in diameter and 6 mm thick). It is also impregnated with cyanoacrylate glue. We coat the external coil with Super Steel Epoxy. The total dimensions of the external coil are 24 mm in diameter and 8 mm in thickness. The electrical characteristic of the coil is a DC resistance of about 200 Ω. Our way of placing the external coil over the internal coil is by taking away one speaker of a headband set and replacing it with the external coil (Fig 1).

External Stimulator. We use a folded aluminum

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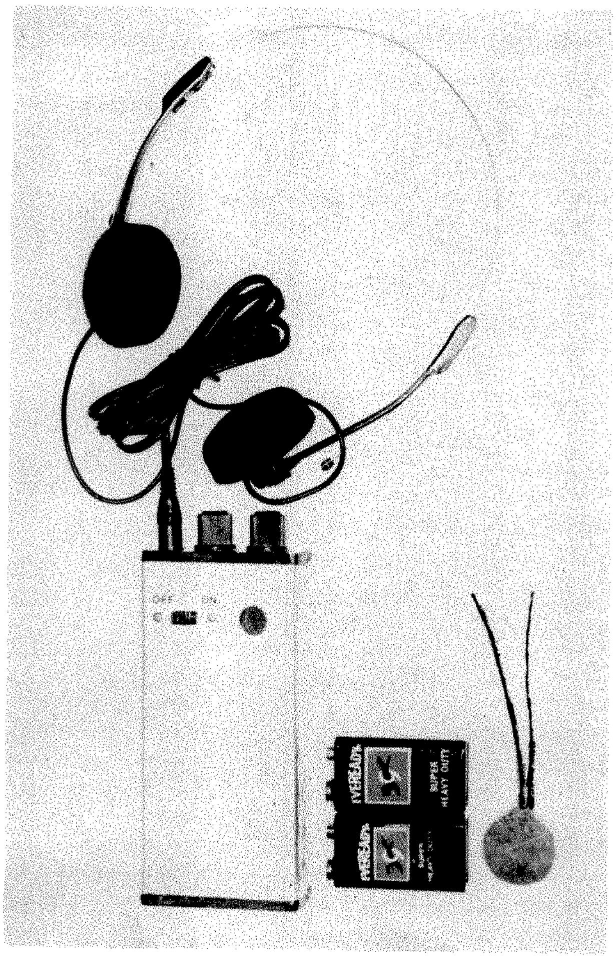

Fig 1. Inexpensive cochlear implant device consisting of internal coil, external coil (in headbands), and external stimulator.

case with dimensions of 11.6 x 5.9 x 2.8 cm. It is powered by two 9-V carbon-zinc batteries. The total weight is 172 g (Fig 1). On the front, there is a condenser microphone and an on-off switch. On the top, there is a volume control knob, a sensitivity control knob, and a plug for the external coil.

Signal Flow. The sound enters the microphone with a preamplifier circuit and is routed to a bandpass filter. At the preamplifier circuit, the total gain from the microphone is controlled by a sensitivity circuit. The 3-dB down-points of the filter section are at 200 and 5,000 Hz. From the output of the filter, the signal is routed to a volume control circuit and then to a driver amplifier. Finally, it is routed to the external coil (Fig 2). The electrical stimulus from the internal coil is of a sine waveform.

The patients felt discomfort when the voltage at the external coil was about 15 V peak-to-peak. With the circuit designed, the clipping level is at 15 V peak-to-peak, with new batteries, as in Fig 3.

Patients are selected for a CI device if it is determined that they have a profound sensorineural hearing loss and gain no benefit from hearing aids. The patients must be able to read and write. They should be postlingually deafened adults who are highly cooperative and in general good health. The ear proposed for implantation must be free from infection and middle ear disorders. The tympanic membrane must be intact. We have implanted devices in six patients since March 26, 1988. Four of them are profoundly deaf from ototoxic drugs, and two from head injuries. All are postlingually deafened adults with poor to good lipreading abilities (ages ranged from 32 to 60 years). We did a promontory stimulation test on all of them to make sure that the hearing pathway beyond the cochlea was still good. We used a transtympanic needle with a biphasic current waveform for the stimulation. The surgical procedures were performed through the facial recess approach to the middle ear as described by House.[5]

After implantation, the auditory performance of these patients was assessed by using pure tone audiometry, speech perception tests, and environmental sounds tests. Speech perception tests consist of 1) spondee words (20 items), 2) consonant-vowel-consonant rhyme discriminations (30 items of initial rhymes and 36 items of final rhymes), and 3) monosyllabic word discriminations (25 items).

The tests were prepared in the Thai phonemic system. Words that are commonly used in northern Thai everyday life were selected for the tests. They were tested for standardization in 10 normal subjects. The index of difficulty is 8.4 (moderately difficult).

Each item was presented by live voice at normal conversational loudness (about 70 dB sound pressure level [SPL]). The tests were performed in a quiet room under two conditions. First was lipreading only and second was lipreading with the CI device. Under both conditions the subject had to repeat each item.

The environmental sounds tests consisted of 10 sounds that are commonly heard in everyday life. All sounds used were readily identified by a group of 10 normal-hearing listeners. The sounds were presented once, and were repeated once if necessary. Subjects were instructed to identify what they heard.

RESULTS

The first patient refused to use the CI device, as she was content with her good lipreading ability. Even after a 1-year follow-up period, she still refused to use it. She had had high expectations and was disappointed about the sound she perceived through the CI device. She did not like the headbands either. Her internal coil, however, has not been removed.

The internal coil of the third patient had to be removed after 4 months of implantation. This was be-

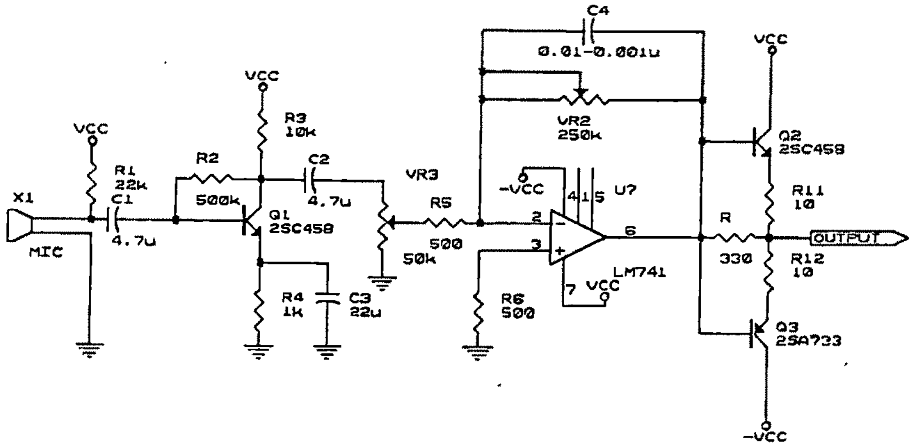

Fig 2. Circuit schematic of external stimulator.

cause he worked with a welding machine that he claimed interfered with his internal coil, although he tried to stay as far away as possible from the machine while he was working with it. Other patients reported that they could perceive sound from an electrical haircutter as loud as the noise picked up from the external processor, but this was not uncomfortable. The welding machine produced only a faint sound for some of them.

The audiologic results were evaluated from four patients. Figure 4 shows the pure tone thresholds of the patients. The preoperative unaided thresholds show almost no residual hearing. With hearing aids, the thresholds of the patients are around 110 dB SPL or poorer. The thresholds obtained with the CI device are around 70 dB SPL.

The patients were able to hear from 100 to 8,000 Hz. The thresholds for hearing were about 3 V peak-to-peak at the external coil. Two 9-V batteries lasted 120 to 150 hours.

The coupling coefficient, as with the House/3M CI device, drops off as the coil separation increases or the two coils are misaligned. When the external coil is placed at an exact point, the headband set will hold it in place and therefore there is no need to be frequently readjusting.

After a month of using the CI device, the patients reported that they could discriminate sounds from various origins such as motorcycles, buses, footsteps, barking dogs, adults, children, men, and women. They also reported that their lipreading abilities were improving with the CI device.

Speech perception tests were conducted as open sets and randomly on the second, fifth, and sixth patients after they had used a new-model external stimulator for 1 month. (The second patient had used an old-model external stimulator that had some distortion for 9 months.) The fourth patient was excluded from the tests because he had poor vision and poor lipreading ability.

On speech perception tests, the lipreading abilities of the second, fifth, and sixth patients were 27.2%, 23.2%, and 25.6%, respectively. The lipreading abilities with the CI were 35.2%, 32%, and 30.4%, respectively.

The results of the environmental sounds tests were 100% for the second, fourth, fifth, and sixth patients.

DISCUSSION

It is becoming apparent that multichannel cochlear implant systems that provide spectral or place information in addition to temporal coding generally provide more useful auditory cues to patients than do single-channel designs.[6-10] We tried to invent an inexpensive CI device, as almost all of our patients are poor. We considered single-channel designs, as they require much less investment and less technology than multichannel ones.

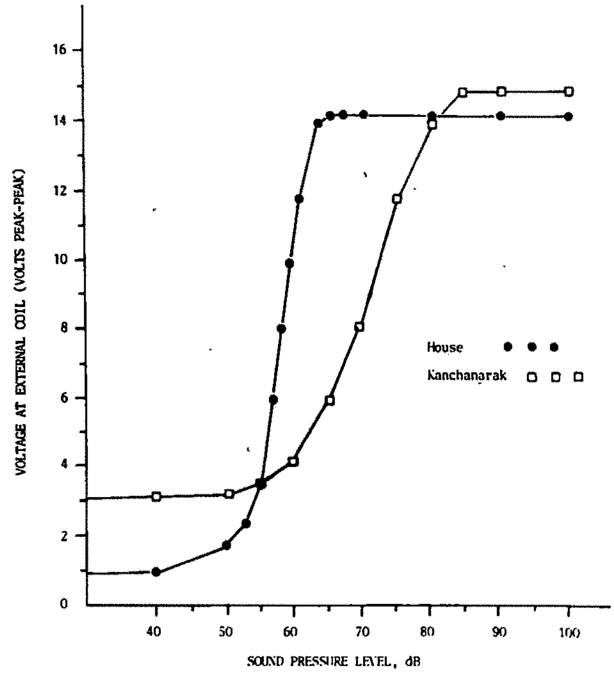

Fig 3. Output of stimulator to external coil in relation to sound pressure level input at microphone, for sine-wave signals.

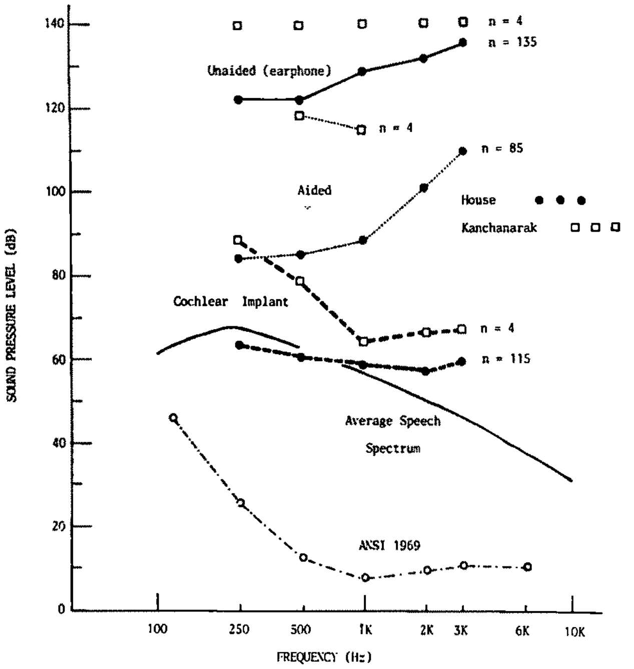

Fig 4. Summary of pure tone thresholds obtained from four patients under earphones and with hearing aids prior to implantation and with cochlear implant device. American National Standards Institute (ANSI) standard for 0 dB hearing level and average long-term speech spectrum are included as references.

We made an inexpensive model by using handmade materials and coating the implanted coil with SSR, SMA, silicone rubber tubing, and 6-0 nylon suture. Using this method, we did not have to invest in high technology and an expensive process for molding medical-grade silicone to coat the coil. The SSR, SMA, and silicone rubber tubing have good biocompatibility.[11] The SMA alone is not strong enough to be used as a coating. The durability and longevity of the implanted coil depend on the coating system. The integrity of the SSR, SMA, silicone rubber tubing, and 6-0 nylon suture coated with SMA is lifelong. Coated with SMA, the silicone adheres to the epoxy coating much more strongly than when coated with silicone elastomer. The potential space between the silicone and epoxy coating for body fluid accumulation should not occur. The SMA is permeable and body fluid can reach the epoxy coating through microporous spaces. The epoxy coating is water-resistant and a good insulator. These characteristics should make the implanted coil lifelong.

We used a ferrite pot core and a headband set, as a samarium cobalt magnet was not available, but the ferrite material is about $100 cheaper. Some patients did not like the model as it made them look like teenagers. We used a folded aluminum case that made the set much less expensive than one with a molded plastic case; the plastic case requires about $1,000 for the mold.

Our speech processing strategy was modified from House's CI device.[1,3,4] We used coils that had larger dimensions and higher resistance. That put the response signal at a low frequency, and it did not require a carrier frequency in coupling of signals from the external coil to the implanted coil. Hence, we removed the amplitude modulator circuit. With these features, the set used less electricity and fewer transistors. Two 9-V carbon-zinc batteries lasted 120 to 150 hours. Danley and Fretz[1] reported that the House device used two 9-V alkaline batteries that lasted 40 to 80 hours. If an alkaline battery lasts seven times longer than a carbon-zinc battery, using the same battery, our CI device lasts about 15 times longer than the House/3M device.

The results are good in four patients. The threshold of our CI device was relatively high at 250 and 500 Hz, owing to the limitation of the condenser microphone. The differences in lipreading scores after the patients had used the external processors for 1 month are not significant, but the scores on environmental sounds tests were 100%. We chose this circuit as we compared external processors constructed from House's circuit and a few circuits that generated sine, square, and biphasic waveforms, and the patients preferred the sine waveform. All of the patients had a fourth grade education. We did not have a good speech rehabilitation program for the patients, and some of them used the external processors for only a few hours a day. This factor might contribute to poor lipreading scores. We are now in the process of designing a new circuit to improve the threshold and performance. We are starting to look at a more standardized way of evaluating the implant. We are also starting to set up a more comprehensive aural rehabilitation program. Further improvement in appearance of this CI device can be achieved by adding a piece of samarium cobalt magnet to hold the coil instead of using a headband set.

CONCLUSION

Manufacturing this CI device is easily accomplished. Coating by hand is the most important step in making it inexpensive. Modifying the coils and circuit makes it use less electricity and fewer transistors. Most of our deaf patients are poor but can afford this model. The authors' aim is to allow all, or as many as possible, to hear.

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SEVENTH INTERNATIONAL SYMPOSIUM ON THE FACIAL NERVE

The Seventh International Symposium on the Facial Nerve will take place June 9-14, 1992, in Cologne, Germany. For further information, please contact the Congress Secretary, Klinik und Poliklinik für HNO, Universität zu Köln, Kongressburo, Joseph-Stelzmann-Strasse 9, D-5000 Köln, Germany; 49-221-478-6190.

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A Conference on Assistive Listening Devices, Tutorials, Applications, and Research will be held June 12-14, 1992, at The University of Iowa, Iowa City, Iowa. For information on registration and accommodation, contact the Conference Center, University of Iowa, Memorial Union, Iowa City, IA 52242; (319) 335-3231, Fax (319) 335-3407. For information on Call for Papers, contact Regina Tisor (319) 356-2471, Fax (319) 356-4547. We have applied for Continuing Education Units from ASHA and HAIC.

Ann Otol Rhinol Laryngol 100:1991

BONE DESTRUCTION MECHANISMS IN CHRONIC OTITIS MEDIA WITH CHOLESTEATOMA: SPECIFIC PRODUCTION BY CHOLESTEATOMA TISSUE IN CULTURE OF BONE-RESORBING ACTIVITY ATTRIBUTABLE TO INTERLEUKIN-1 ALPHA

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To clarify specific mechanisms underlying cholesteatoma-induced bone destruction, surgical specimens of middle ear inflammatory granulation tissue with or without cholesteatoma were maintained in vitro and the bone-resorbing activity in their culture supernatants was analyzed by means of calcium release from mouse calvaria. Almost the same levels of bone-resorbing activity and prostaglandin (PG) E2 were found in the supernatants of both types of tissue. By contrast, aural polyp tissue yielded hardly any such activity or PGE2. Under the influence of indomethacin, however, only tissue with cholesteatoma produced considerable bone resorption activity, whereas PGE2 production was suppressed completely. Such activity in the cholesteatoma culture supernatant was not due to contamination of endotoxin and proved to be blocked by the introduction of anti–interleukin (IL)–1α antibody into the calvarial assay system. Anti–IL-1β antibody had no effect on such activity. Interleukin-1α was detected only in cholesteatoma tissue culture supernatants by means of enzyme-linked immunosorbent assay and by bioassay. These data suggest that the bone destruction in otitis media with cholesteatoma may be attributed to IL-1α in addition to PGE2.

KEY WORDS — bone-resorbing activity, interleukin-1, middle ear cholesteatoma, prostaglandin E2.

INTRODUCTION

Chronic otitis media (COM) is an inflammatory disease clinically characterized by bone destruction. In COM with cholesteatoma, bone damage is more severe than in simple COM without cholesteatoma. For example, loss of auditory ossicles was found in about 80% of the patients suffering from COM with cholesteatoma, while the rate was only 10% to 20% for those with simple COM without cholesteatoma.[1]

Many reports have dealt with the specific mechanisms of cholesteatoma-induced bone damage and several possible explanations for such damage have been proposed; eg, bone destruction is induced by the pressure on bone from swollen tissue of cholesteatoma[2] and by endotoxin[3] or acid products of bacteria in the debris of cholesteatoma.[1] The enzymes such as collagenase and acid hydrolases that can be produced by granulation tissue have also been suggested as causative factors.[4,5] There have been few investigations, however, concerning the possibility of production by cholesteatoma tissue of endogenous mediators inducing bone resorption. Only one examination of this problem, by Bernstein et al,[6] suggested the participation of soluble factors such as prostaglandin (PG) E2 in bone resorption of COM, and further study has not been done.

Recently, various growth factors and cytokines have been demonstrated to have activity that induces bone resorption in vitro and in vivo. Interleukin (IL)-1α and IL-1β are two of the most active substances.[7] Concerning IL-1β, Dewhirst et al[8] proposed that it was identical to the so-called osteoclast-activating factor and Miyasaka et al[9] reported that it was produced in inflamed loci such as rheumatoid pannus. By contrast, however, Robinson et al[10] reported that bone-resorbing activity produced by such tissue was almost always attributable to PGE. Thus, the roles of these substances such as IL-1 in bone resorption of inflammatory diseases is still uncertain, and it is still unclear which mediators are responsible for bone damage associated with middle ear cholesteatoma.

In this report, therefore, we maintained tissues in vitro that were surgically obtained from patients with otic inflammatory diseases including COM with or without cholesteatoma, and we attempted to analyze the bone resorption–inducing activity produced by these tissues by means of calcium liberation from mouse calvaria in vitro. The relationship between the activity and clinically observed bone damage in COM is also discussed.

MATERIALS AND METHODS

Preparation of Culture Supernatants of Tissues From Patients With Otic Inflammation. Inflammatory tissues were surgically obtained at the time of middle ear and mastoid surgery from COM patients with cholesteatoma and without cholesteatoma. The tissue was cultured as described below accord-

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REPRINTS — Atsushi Kurihara, PhD, Dept of Otolaryngology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan.

ing to the method in the literature,[10,11] with slight modification.

Cholesteatoma tissue, together with subepithelial granulation tissue from which keratin debris was removed before surgery, was stored immediately after resection until processing at 4°C in Dulbecco's minimum essential medium (DMEM; Nissui Pharmaceutical Co Ltd, Tokyo, Japan) that was supplemented with HEPES (25 mmol/L) and antibiotics (streptomycin sulfate 0.1 mg/mL, penicillin G potassium 0.1 U/mL, amphotericin B 0.25 μg/mL). After washing with DMEM, the tissue was divided into two equal portions by weight, cut into small pieces (2 mm^3), and precultured for 1 hour with fresh DMEM in 35-mm Falcon dishes (Becton Dickinson and Co, Lincoln Park, NJ). One milliliter of medium per 30 mg of tissue was used. Medium was then replaced with fresh DMEM and both portions of the tissue were further cultured for 5 days. One portion of the tissue was maintained under the influence of indomethacin (10 μg/mL). Indomethacin was dissolved in ethanol and diluted with DMEM. Ethanol was added to the plain DMEM at a concentration of 0.1% in which the other portion of the tissue was maintained. At 24 and 72 hours, culture supernatants were replaced with fresh medium with or without indomethacin. At the end of culturing, culture supernatants were again collected and all supernatants were stored at −80°C. When bacterial or fungal contamination appeared, the supernatant was discarded.

Because of the low volume of the supernatant, supernatants collected from three tissues at various times were pooled for the groups that were treated with or without indomethacin and used for analysis as described below.

Granulation tissue that was formed in the mastoid antrum of the patients with simple COM without cholesteatoma and the aural polyps that developed in the eardrums of patients with COM were processed as described above, and conditioned media were also pooled.

Assay of Bone-Resorbing Activity. Bone-resorbing activity was assessed biologically by means of a mouse calvarial culture system. Pregnant mice (ICR strain) were purchased from Charles River Japan Inc, Kanagawa, Japan, and calvaria were removed from newborn mice at 5 days of age. A pair of bones, each 4 mm in diameter, were punched out, one from each side of the calvaria, and each bone was preincubated with 0.5 mL of BGJb medium (Sigma Chemical Co, St Louis, Mo) supplemented with streptomycin and penicillin in a Coster 48-well plate (Data Package Corp, Cambridge, Mass). Twenty-four hours later, the medium was replaced with fresh BGJb medium without antibiotics but containing pooled samples of the tissue culture supernatant, and the bone was further incubated for 3 days. Then the concentration of total calcium in the medium was determined by an *o*-cresolphthalein complexone method (Ca-C test; Wako Pure Chemical Industries, Tokyo, Japan). An increase in calcium content was regarded as being the result of bone resorption.

In order to characterize the bone-resorbing activity, the following substances were applied to the calvarial system together with tissue culture supernatant: anti-recombinant human IL-1α mouse monoclonal antibody (Dainippon Pharmaceutical Co Ltd, Osaka, Japan), anti-recombinant human IL-1β rabbit polyclonal antibody (Cistron Biochemistry, Pine Brook, NJ), polymyxin B sulfate (Sigma Chemical Co), and human calcitonin (CT; Peptide Institute, Inc, Osaka, Japan). These substances were dissolved in phosphate-buffered saline (PBS) and diluted with the medium for use. Control media contained PBS at concentrations of 1% to 5%. A preliminary experiment in our laboratory revealed that over 5% of PBS spontaneously increased calcium release from calvaria.

Recombinant human IL-1α and IL-1β (mouse thymocyte co-mitogenic activity: 2.26 x 10^7 and 2 x 10^7 U/mg protein, respectively; kindly supplied respectively by Dainippon Pharmaceutical Co Ltd and by Otsuka Pharmaceutical Co Ltd, Tokushima, Japan), lipopolysaccharide (LPS) derived from *Escherichia coli* (055:B5; Difco Laboratories, Detroit, Mich), parathyroid hormone (PTH [human 1-34]; Peptide Institute, Inc), and PGE2 (Funakoshi Chemical Co, Tokyo, Japan) were also applied to the calvarial system as standard materials inducing bone resorption.

Data were expressed by mean values (± SE) of six bones, and statistical significance between activities was calculated by the F test.

Determination of Contents of PGE2, IL-1, and Endotoxin in Tissue Culture Supernatants. Pooled samples of the supernatants were diluted with assay buffer, and the concentration of PGE2 was measured directly without extraction by a radioimmunoassay kit specific for PGE2 (NEN Products; E. I. du Pont de Nemours & Co, North Billerica, Mass). It was preliminarily certified that the value assayed directly was identical with that assayed after extraction of PG fraction with the aid of Bond-Elut columns (C-18 and Silica; Analytichem International, Harbor City, Calif). Accuracy of the data obtained without extraction was confirmed by demonstrating the complete recovery of PGE2 added exogenously into a sample and the straight-line relationship between the values of a sample with serial dilutions and dilution ratio. It was also confirmed that the antibody against PGE2 in the kit hardly cross-reacted with PGF2α, thromboxane B2, 6-keto-PGF1α, and PGD2.

The levels of IL-1α and IL-1β in pooled samples were examined with specific enzyme-linked immunosorbent assays as described by Tanaka et al.[12] In brief, pooled samples were incubated in a Linbro

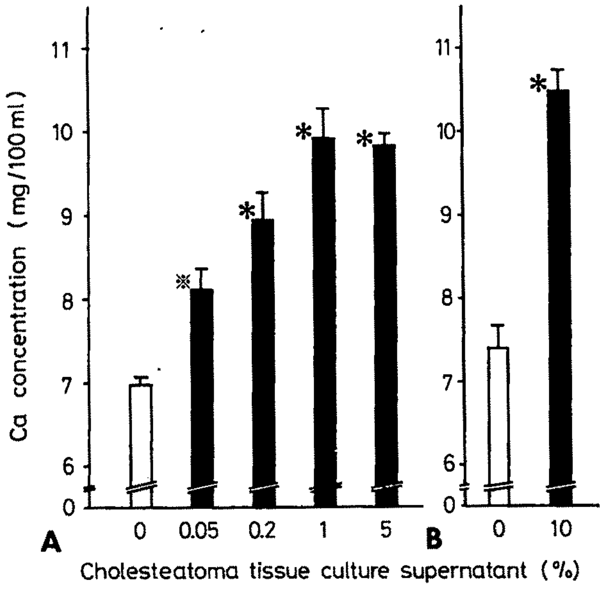

Fig 1. Concentration dependency of calcium (Ca) release from calvaria by cholesteatoma tissue culture supernatant. Culture medium conditioned by cholesteatoma tissue for 72 hours without indomethacin was introduced to calvarial culture system, and Ca levels in calvarial media were estimated after incubation for 3 days. Control values, shown by open columns, were obtained by culturing calvaria with 5% of vehicle fresh DMEM. Statistical significance against control is indicated by ※ ($p<.01$) and * ($p<.001$). Experiments on calvarial system were done twice with same culture medium at indicated concentrations, and data are shown. A) Experiment 1. B) Experiment 2.

96-well plate that was precoated with anti-human recombinant IL-1α or IL-1β mouse monoclonal antibody. Twenty-four hours later, the wells, were washed with PBS, blocked with a solution of bovine serum albumin, and treated successively with anti-human recombinant IL-1α or IL-1β rabbit antiserum and peroxidase-labeled anti-rabbit IgG goat antibodies. Then substrate (*o*-phenylenediamine) solution for peroxidase was incubated in the wells and absorbance of the solution was measured at 492 nm. The lowest detectable levels of IL-1α and IL-1β were respectively 10 and 100 pg/mL. Anti–IL-1α and -IL-1β antibodies used in the assay did not cross-react with each other nor with human IL-2, tumor necrosis factor, granulocyte and/or monocyte colony-stimulating factor, interferon-α, and interferon-γ. The assay systems were commercially available from Otsuka Pharmaceutical Co, in whose laboratory part of the assay was performed. A bioassay of IL-1 as a lymphocyte-activating factor was also done by means of a mouse thymocyte co-stimulator assay.[13] Nonadherent thymocytes obtained from 4- to 8-week-old mice (C3H/He strain; Charles River Japan Inc) were cultured in RPMI-1640 medium supplemented with fetal bovine serum and phytohemagglutinin (PHA; Difco Laboratories). Pooled samples were diluted 1:4 with the RPMI-1640 medium and applied to thymocyte culture. Three days later, enhancement by the samples of mouse thymocyte proliferation induced with PHA was assessed by radioactivity of the cells, which were pulse-labeled with ^{3}H-thymidine.

Endotoxin contamination of the samples was evaluated with the chromogenic endotoxin-specific assay by using recombined limulus coagulation enzymes (Endospecy test; Seikagaku Kogyo Co Ltd, Tokyo, Japan).[14] Limulus amebocyte lysate, from which (1-3)-β-D-glucan-sensitive factor (factor G) was eliminated in order that activation of the limulus-clotting enzyme be triggered only by the endotoxin-sensitive factor (factor C), and the chromogenic substrate (Boc-Leu-Gly-Arg-*p*-nitroanilide) for the clotting enzyme in the lysate were incubated with aliquots of the samples at 37°C for 30 minutes. Then absorbance of the mixture at 405 nm was measured. The lowest detection limit of endotoxin in this system was 1 pg/mL.

RESULTS

Bone-Resorbing Activity in Culture Supernatants. In order to decide the suitable concentration of the tissue culture supernatant for the determination of its bone-resorbing activity, the effect of the supernatant on mouse calvarial bone was preliminarily examined by adding cholesteatoma-conditioned medium to the calvarial system at several concentrations. The results (Fig 1) indicate that bone resorption took place in a concentration-dependent manner and was maximum at 1% to 10% of the supernatant. In the following experiments, therefore, supernatant samples were applied at a concentration of 5% to the calvarial assay system.

From the supernatants of cholesteatoma tissue culture, four sets of pooled samples were made and their bone-resorbing activity was assayed. Since the four corresponding sets of results had substantially the same tendency, two typical cases are shown in Fig 2A,B. Similarly, two sets and a single set of pooled samples were prepared, respectively, from conditioned media of granulation tissue in cases of simple COM without cholesteatoma and from conditioned media of aural polyp tissue. The two sets of results in cases of simple COM without cholesteatoma were almost the same; the data of one are shown in Fig 2C. Figure 2D represents the results concerning aural polyp tissue.

Under the indomethacin-free condition, intense bone-resorbing activity was found to be produced by cholesteatoma tissue as well as by simple COM tissue without cholesteatoma throughout the periods of culturing, as revealed with closed columns in Fig 2A-C. Calcium concentrations in the media of calvaria treated with both COM tissue culture supernatants were significantly elevated at 2 to 5 mg/100 mL above those in control calvarial media. In contrast to the cases of COM, hardly any activity was detected (Fig 2D) in the samples of aural polyps

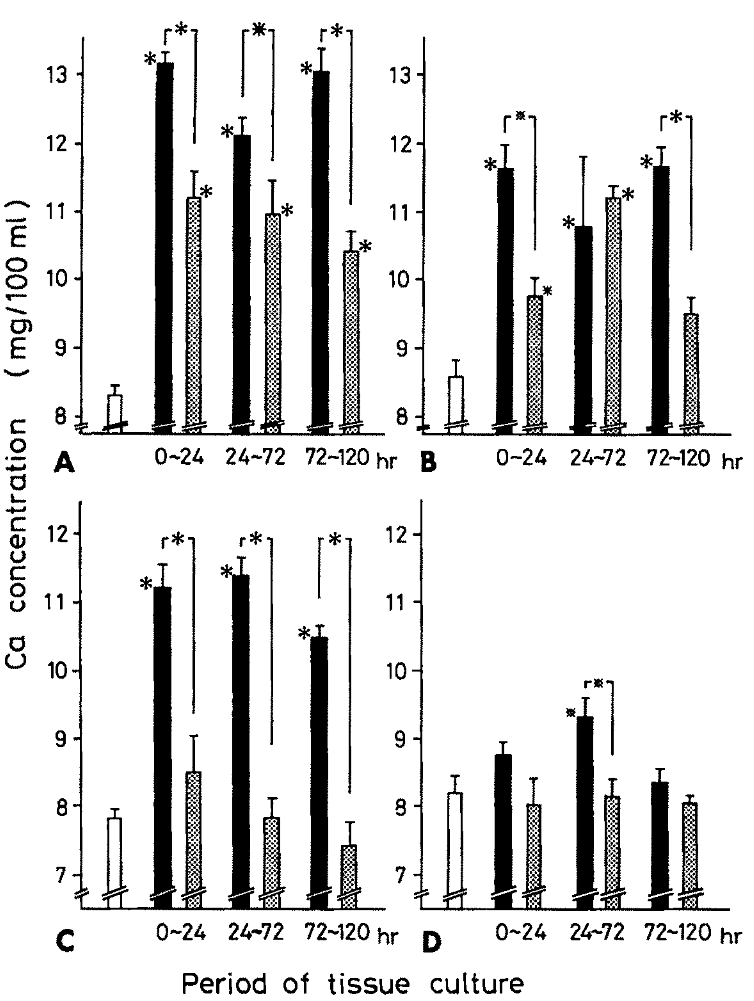

Fig 2. Bone-resorbing activity in culture supernatants of tissue. All tissues were cultured for 5 days, and culture media conditioned for periods of 0 to 24 hours, 24 to 72 hours, and 72 to 120 hours were subjected to calvarial assay. Dotted and closed columns represent calcium (Ca) release from calvaria induced by supernatants prepared with or without indomethacin, respectively. Open columns represent Ca release under control condition. Statistical significance against control is indicated by ✱ ($p < .05$), ⁎ ($p < .01$), and ∗ ($p < .001$) at top of columns. Statistical difference between treatment with or without indomethacin is indicated in panels with same symbols. A,B) Cholesteatoma otitis media tissue. C) Granulation tissue in cases of simple chronic otitis media without cholesteatoma. D) Aural polyp tissue.

that were not accompanied clinically with bone destruction.

Figure 2 also shows the effect of indomethacin on the production of bone-resorbing activity. As shown by the dotted columns, depletion of the activity under the influence of indomethacin was found to be nearly complete in the cases of simple COM without cholesteatoma (Fig 2C) but only partial in the cholesteatoma tissue; ie, calcium contents in the cholesteatoma tissue culture supernatant–treated calvarial media were still significantly higher at 1 to 2.5 mg/100 mL than those in control calvarial media. This finding indicates that considerable activity was produced only by the tissues of COM with cholesteatoma even under the influence of indomethacin (Fig 2A,B).

Prior to the above-described examinations, the effect of indomethacin on bone resorption was determined because this drug was contained in supernatant samples. As a result, it was confirmed that no detectable influence on bone resorption induced by PGE2 (10 ng/mL), IL-1α (2 U/mL), IL-1β (2 U/mL), and cholesteatoma culture supernatant prepared without indomethacin was exerted by the drug at 0.5 μg/mL, which corresponded to the concentration in the calvarial culture medium containing the supernatant samples prepared with 10 μg/mL of indomethacin (Table 1).

Production of PGE2 by Tissues. Concentrations of PGE2 in all seven sets of pooled samples were measured by radioimmunoassay and the results are

TABLE 1. INERTNESS OF INDOMETHACIN ON BONE RESORPTION INDUCED BY IL-1α, IL-1β, PGE2, AND CHOLESTEATOMA TISSUE CULTURE SUPERNATANT

Condition of Calvarial Culture	*Calcium Concentration (mg/100 mL)*	*Significance vs Control*
Control	8.66±0.14	—
IL-1α (2 U/mL)	11.19±0.34	p<.001] NS
With indomethacin*	11.09±0.40	p<.001
IL-1β (2 U/mL)	11.43±0.46	p<.001] NS
With indomethacin*	10.64±0.63	p<.001
PGE2 (100 ng/mL)	10.60±0.54	p<.001] NS
With indomethacin*	11.40±0.38	p<.001
Control	6.82±0.14	—
Cholesteatoma supernatant†	11.52±0.39	p<.001] NS
With indomethacin*	11.22±0.39	p<.001

NS — not significant.

*Indomethacin was introduced at concentration of 0.5 μg/mL to calvarial system together with IL-1α, IL-1β, PGE2, and cholesteatoma tissue culture supernatant.

†Culture supernatant (24 to 72 hours) of cholesteatoma tissue was prepared without indomethacin.

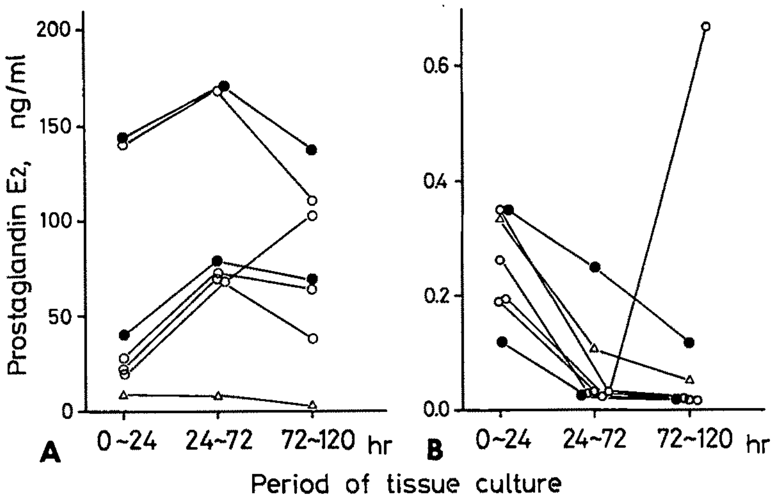

Fig 3. Prostaglandin E2 levels in pooled samples of culture media conditioned by cholesteatoma tissue (○), granulation tissue in cases of simple chronic otitis media without cholesteatoma (●), and aural polyps (△) for indicated periods. A) Under indomethacin-free condition. B) Under influence of indomethacin.

summarized in Fig 3. As shown in Fig 3A, PGE2 at levels ranging from 20 to 170 ng/mL was found in both cholesteatoma tissue and simple COM granulation tissue culture supernatants when they were prepared without indomethacin, and no difference was found between the two tissues. By contrast, the content in the culture supernatant of aural polyps was relatively low (<10 ng/mL), in parallel with their bone-resorbing activity.

The addition of indomethacin to the tissue culture strongly depressed PGE2 production by COM tissue with or without cholesteatoma by at least 1%, and there was little difference in PGE2 levels among the three kinds of tissue. The highest PGE2 level in Fig 3B (0.67 ng/mL) is far less than the levels in the indomethacin-free supernatant of aural polyp tissue (Fig 3A), in which bone-resorbing activity was hardly detected (Fig 2D). However, the activity derived from cholesteatoma, especially at the second phase (24 to 72 hours) of culture, seems to be affected only partially by indomethacin (Fig 2A,B). Such activity was analyzed in the following experiments.

Characterization of Cholesteatoma-Derived Bone-Resorbing Activity. Because of the possibility of contamination in the culture supernatants of endotoxin that might be derived from tissues obtained from infected lesions, concentrations of endotoxin in one of the pooled samples of cholesteatoma tissue were determined preliminarily and endotoxin at 290 to 500 pg/mL was detected (data not shown). Therefore, it was confirmed, with the aid of polymyxin B sulfate, that endotoxin was not related to the bone-resorbing activity derived from cholesteatoma tissue. As shown in Fig 4, the addition to the calvarial culture system of polymyxin B sulfate at a concentration of 10 μg/mL, which could inhibit bone-resorbing activity induced by LPS maximally at 10 ng/mL, had no effect on the activity in cholesteatoma tissue culture supernatant prepared with or without indomethacin; neither did it have an effect on IL-1α– and IL-1β–induced bone resorption.

The supernatant samples of cholesteatoma tissue culture did not induce calcium release from calvarial bone that was devitalized by repeated freezing and thawing (Table 2). This finding indicates that the bone-resorbing activity is not due to a nonspecific physicochemical influence on bone. In order to demonstrate the dependence on osteoclasts of calcium release from living calvaria by the cholesteatoma tissue culture supernatant, the effect of calcitonin, which has been reported to inhibit osteoclast activation,[15] on bone-resorbing activity was evaluated. Human calcitonin was found to inhibit bone resorption induced not only by PTH, PGE2, and IL-1β, but also by cholesteatoma tissue culture supernatant (Fig 5).

Figures 6 and 7 show results of the introduction of anti–IL-1 antibodies to the calvarial assay system to clarify the participation of IL-1α and IL-1β in the bone-resorbing activity produced by indomethacin-treated cholesteatoma tissue. When antibody solution against the α type of IL-1 was serially diluted with the calvarial medium, IL-1α–induced bone resorption was inhibited by the antibody in a concentration-dependent manner (Fig 6A). On the basis of this result, anti–IL-1α antibody solution was applied at a dilution ratio of 1:120 to the calvarial assay system together with cholesteatoma tissue culture supernatant. This application completely suppressed cholesteatoma-derived bone-resorbing activity in tissues prepared with indomethacin, whereas those prepared without indomethacin were not influenced (Fig 6C). This antibody solution had no effect on the bone-resorbing activity of IL-1β, nor on that of PGE2 and PTH (Fig 6B). This finding indicates that blockade by the antibody was specific to IL-1α–induced bone resorption. In contrast, anti–IL-1β antibody, even when an undiluted solution of the antibody was directly added to the calvarial system (final dilution ratio 1:20), exerted no influence on the activity produced by cholesteatoma, although the bone-resorbing activity of IL-1β was completely suppressed (Fig 7).

Detection of IL-1α in Culture Supernatant of

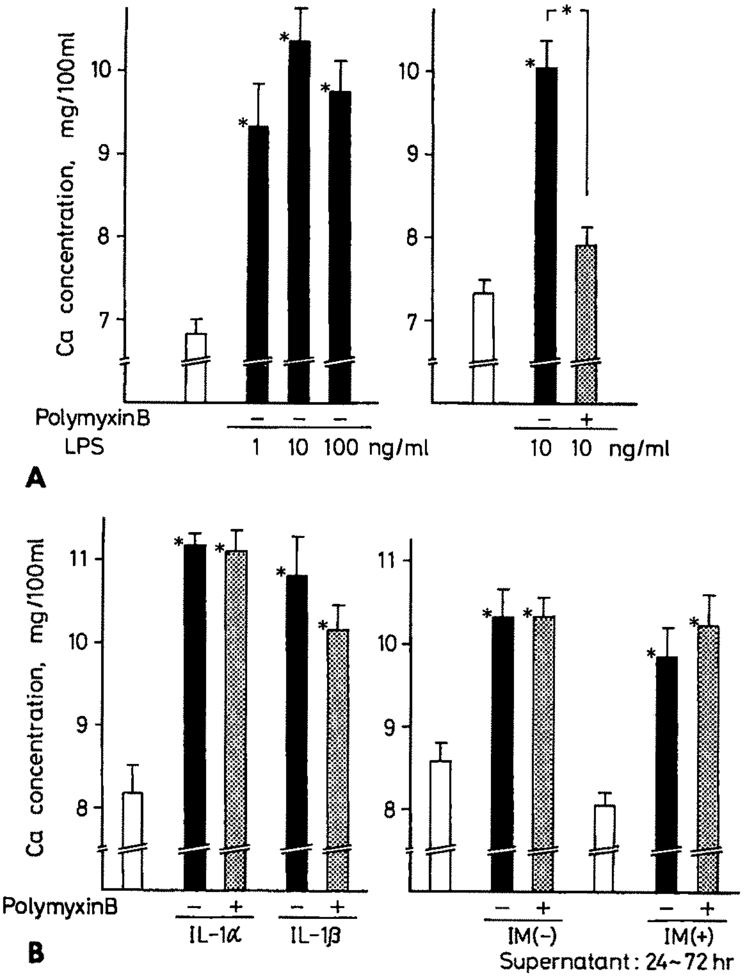

Fig 4. Effect of polymyxin B sulfate on bone-resorbing activity. Control values, shown by open columns, were obtained by culturing calvaria with vehicle solutions. Statistical significance against control is indicated by * ($p<.001$) at top of columns. A) Bone-resorbing activity of endotoxin (LPS) at indicated concentrations and blockade by polymyxin B sulfate (10 μg/mL). Significant difference between groups is indicated by * with brackets ($p<.001$). B) Inertness of polymyxin B sulfate (10 μg/mL) on bone-resorbing activity of IL-1α (2 U/mL), IL-1β (2 U/mL), or cholesteatoma tissue culture supernatant (24 to 72 hours) prepared with or without indomethacin. Treatment of tissue with or without indomethacin is indicated by IM(+) or IM(−), respectively.

Cholesteatoma. Enzyme-linked immunosorbent assay was used for the determination of IL-1α concentrations in all samples, and IL-1α was found only in cholesteatoma-derived supernatants but not detected in samples of the other two kinds of tissue. As shown in Fig 8, IL-1α levels in cholesteatoma tissue culture supernatants increased from 1 to 3 days of culture maximally at 270 pg/mL, and then decreased. No obvious effect of indomethacin on the levels of IL-1α was apparent. Interleukin-1β was not detected in any of the samples by means of enzyme-linked immunosorbent assay, probably because the lowest detectable level of IL-1β was higher than that of IL-1α in this assay method. Therefore, the biologic activity of IL-1 as a lymphocyte-activating factor in the supernatants of tissues cultured with indomethacin was characterized by means of murine thymocyte co-stimulator assay. As shown in Table 3, ^{3}H-thymidine incorporation by the cells was enhanced 5- to 38-fold by cholesteatoma tissue culture supernatants, and over 80% of the enhancement was blocked by anti–IL-1α antibody, while inhibition due to anti–IL-1β antibody was only 23% to 29%. Thus, it was indicated that IL-1α levels predominated over those of IL-1β.

DISCUSSION

There have been several studies on production and release of bone resorption–inducing substances by inflamed tissues like rheumatoid synovial pannus.[9,10] Concerning middle ear inflammation, Bernstein et al[6] reported that bone-resorbing activity was detected in culture of cholesteatoma tissue as well as in culture of granulation tissue without cholesteatoma and in middle ear effusion. However, the relationship between the results and clinical conditions of bone resorption has not been fully discussed in the literature. Therefore, one of the aims of our study was to elucidate this problem by using

TABLE 2. CALCIUM RELEASE FROM LIVING BONE BUT NOT FROM DEAD BONE INDUCED BY CULTURE SUPERNATANT OF CHOLESTEATOMA

	Living Bone		*Dead Bone**	
Condition of Calvarial Culture	*Calcium (mg/100 mL) ±SE*	*Versus Control*	*Calcium (mg/100 mL) ±SE*	*Versus Control*
Control	6.69±0.14	—	7.48±0.06	—
Cholesteatoma supernatant†				
0 to 24 h	8.94±0.18	$p<.01$	7.10±0.15	NS
24 to 72 h	9.17±0.19	$p<.01$	7.04±0.15	NS
72 to 120 h	8.86±0.29	$p<.01$	7.30±0.25	NS

NS — not significant.

*Mouse calvarial bone was devitalized by repeated (three) freezing and thawing and then treated similarly as living bone.

†Cholesteatoma tissue culture supernatant was prepared without indomethacin at indicated time steps.

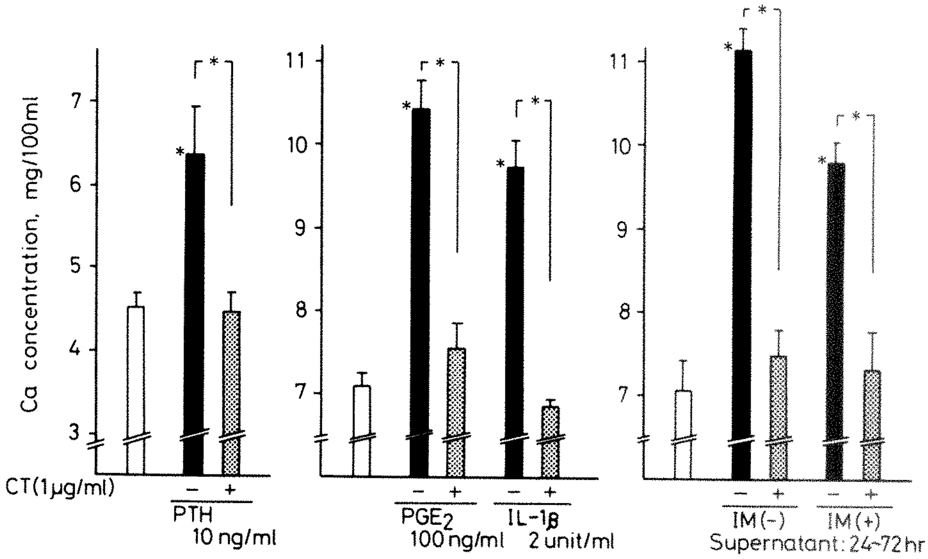

Fig 5. Effect of calcitonin (CT; 1 μg/mL) on bone resorption induced by parathyroid hormone (PTH; 10 ng/mL), PGE2 (100 ng/mL), IL-1β (2 U/mL), and 24- to 72-hour culture supernatant of cholesteatoma tissue. Preparation of supernatant with or without indomethacin is indicated by IM(+) or IM(−), respectively. Open columns and statistical differences are shown similarly as in Fig 4.

three kinds of tissues as described in the text. Damage and loss of bone are clinical features of COM, but aural polyps are thought to be unrelated to these phenomena.[16] Corresponding to these clinical observations, bone-resorbing activity was found to be strongly produced by COM tissues both with and without cholesteatoma under the control condition, but such activity produced by aural polyp tissue was slight (Fig 2). However, no difference between cholesteatoma tissue and granulation tissue without cholesteatoma appeared under the control condition, while bone destruction is clinically associated more frequently and severely with COM with cholesteatoma than with simple COM without cholesteatoma. This may be accounted for in at least two ways as follows.

1. The calvarial bioassay is a semiquantitative method for detection of bone-resorbing activity. Although supernatant samples were applied at the concentration of 5% into the calvarial system in order to widely detect bone-resorbing activity, exact quantification of the activity was thought to be somewhat difficult because, as shown in Fig 1, calcium liberation from calvaria reached a plateau when cholesteatoma supernatant was added at the concentration of 1% to 10%.

2. Cholesteatoma specimens were not thought to be obtainable in the active phase of the disease, because the surgical procedure was generally preceded by attempts to reduce inflammatory reactions.

Bernstein et al[6] also did not find any qualitative difference in production of bone-resorbing activity between these two disease states, but they speculated that such activity was partially attributable to PGE2. In the present experiment, PGE2 was indeed detected, parallel with bone-resorbing activity, at much higher levels in the culture supernatants of granulation tissue with and without cholesteatoma than in the supernatant of polyp tissue (Fig 3A). We therefore introduced indomethacin, a typical cyclooxygenase inhibitor, into the tissue culture to inhibit PG synthesis by the tissues. Consequently, it was found that only cholesteatoma tissue culture supernatant prepared under the influence of indomethacin possessed significant bone-resorbing activity (Fig 2A,B), whereas PGE2 levels were diminished completely in all tissue culture supernatants (Fig 3B) and bone-resorbing activity was not detected in culture supernatant of tissue in cases of simple COM without cholesteatoma (Fig 2C). These results indicate that bone-resorbing activity derived from granulation tissue without cholesteatoma is mostly attributable to PGE2 and that the activity of tissue that includes cholesteatoma is due not only to PGE2 but also to another factor(s). This suggests that another factor(s) produced even under the influence of indomethacin is responsible for severe bone damage accompanying cholesteatoma. Although Chambers et al[17] reported suppression of activity of isolated osteoclasts by PGE2, it has been shown by Akatsu et al[18] that differentiation and maturation of precursor cells into osteoclasts was stimulated by PGE2. Prostaglandin E2–induced resorption of mouse calvaria in a dose-dependent manner was also confirmed in our laboratory (data not shown).

The present study also aimed to characterize cholesteatoma-derived bone-resorbing activity. Figure 5 indicates that calcium release from calvarial bone is not accelerated by the influence of calcitonin, which has been demonstrated to cause osteoclasts and their precursor cells to be static. This suggests that a cholesteatoma tissue–derived substance(s) induces bone resorption through maturation and/or activation of osteoclasts in bone tissue. Several pathologic studies have described the absence of osteoclasts in bone lesions of COM and have suggested the involvement of osteoclast-independent mechanisms in bone damage.[5,19,20] However, activated osteoclasts have been reported to have a relatively short life span, which might cause the disappearance of osteoclasts in surgical specimens obtained

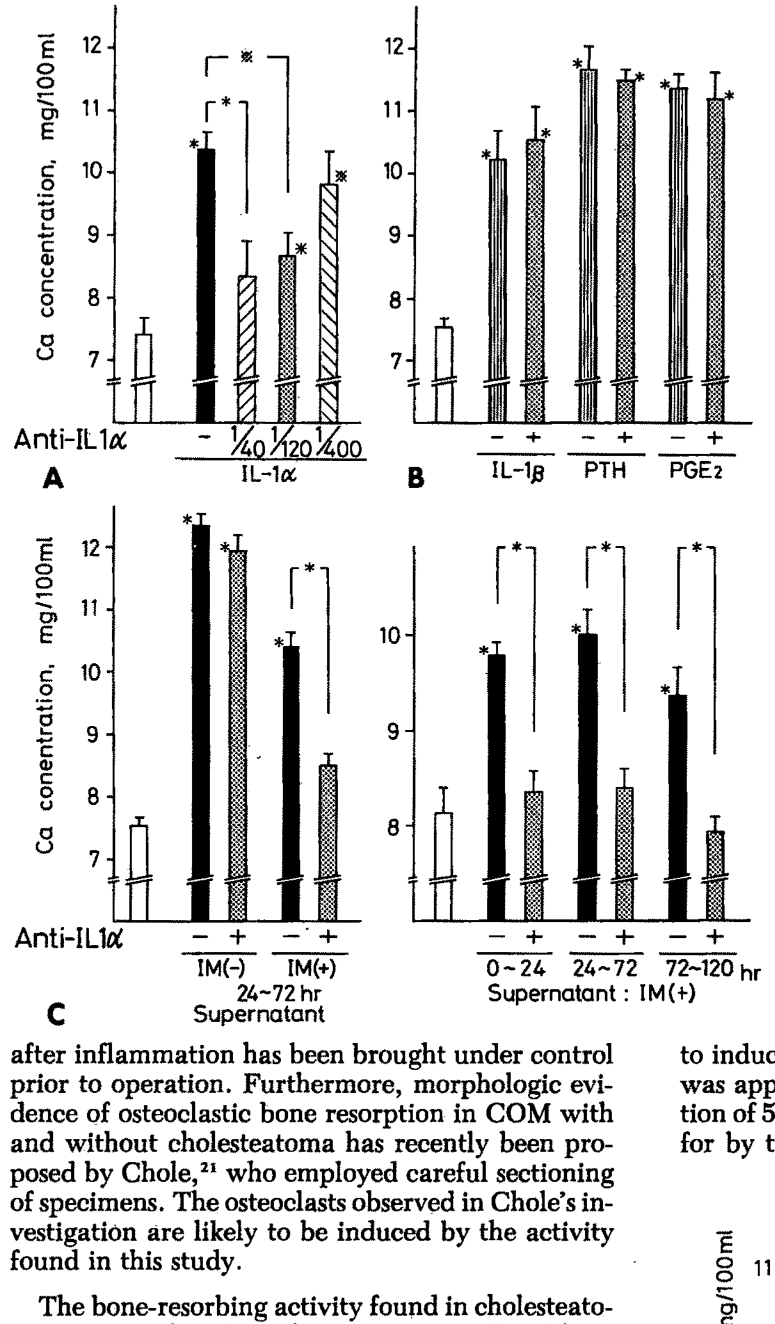

Fig 6. Effect of anti–IL-1α antibody on bone-resorbing activity. Control values are shown by open columns. Statistical significance against control is indicated by ✱ ($p<.05$), ✻ ($p<.01$) and * ($p<.001$) at top of columns, and statistical difference between groups is indicated by ✻ ($p<.01$) and * ($p<.001$) with brackets. A) Anti–IL-1α antibody solution was diluted at indicated ratios with calvarial medium containing IL-1α (2 U/mL). B) Anti–IL-1α antibody solution was introduced at dilution ratio of 1:120 to calvarial system with IL-1β (2 U/mL), PTH (10 ng/mL), and PGE2 (100 ng/mL). C) Bone-resorbing activity of culture media conditioned by cholesteatoma tissue for indicated periods was estimated under presence of anti–IL-1α antibody. Preparation of conditioned media with or without indomethacin is indicated by IM(+) or IM(−), respectively. Anti–IL-1α antibody solution was diluted similarly as in B.

after inflammation has been brought under control prior to operation. Furthermore, morphologic evidence of osteoclastic bone resorption in COM with and without cholesteatoma has recently been proposed by Chole,[21] who employed careful sectioning of specimens. The osteoclasts observed in Chole's investigation are likely to be induced by the activity found in this study.

The bone-resorbing activity found in cholesteatoma tissue with indomethacin is not attributable to contamination of bacterial endotoxin, as shown by the ineffectiveness of polymyxin B sulfate on the activity (Fig 4). Furthermore, antibody that could specifically neutralize IL-1α activity was proved to block bone-resorbing activity of cholesteatoma tissue culture supernatant prepared with indomethacin (Fig 6). Also, IL-1α was detected only in the culture supernatant of cholesteatoma tissue (Fig 8). These results indicate that IL-1α is responsible for the activity derived from indomethacin-treated cholesteatoma tissue. However, significant calcium release in our calvarial assay system was induced with a recombinant form of human IL-1α at 1 U/mL (45 pg/mL; data not shown). Therefore, levels of IL-1α in the supernatants seem to be insufficient to induce bone resorption, because the supernatant was applied to the calvarial system at a concentration of 5%. This result might be partially accounted for by the difference in bone-resorbing activity of

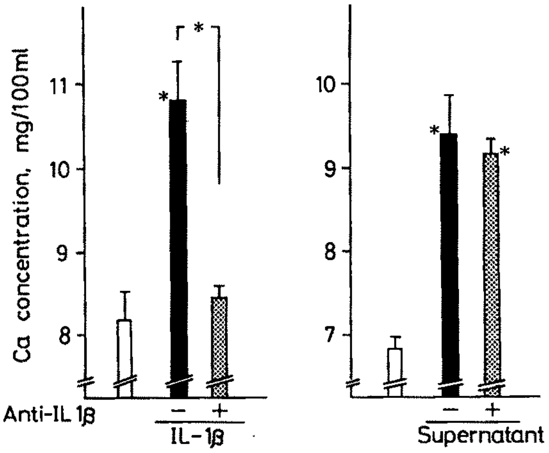

Fig 7. Effect of anti–IL-1β antibody on bone-resorbing activity of IL-1β (2 U/mL) and 24- to 72-hour supernatant of cholesteatoma tissue culture prepared with indomethacin. Anti–IL-1β antibody solution was applied to calvarial system at final dilution ratio of 1:20. Other notations are same as in Fig 6.

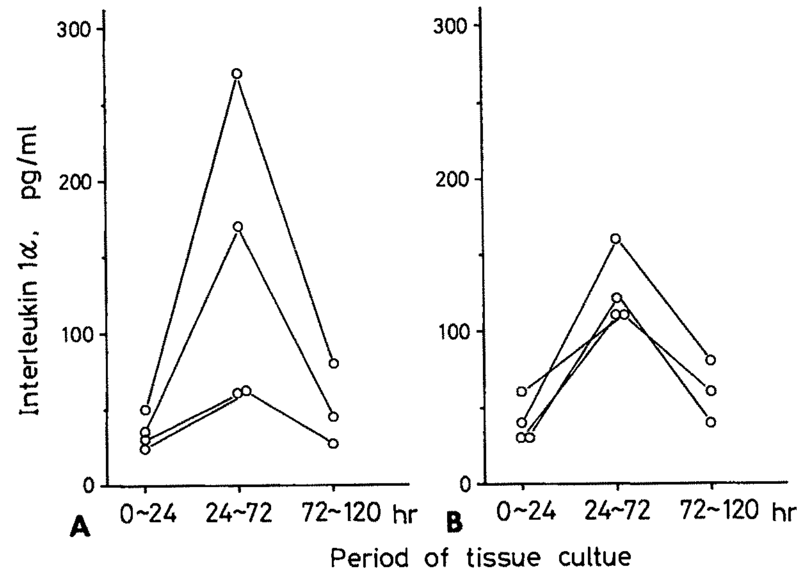

Fig 8. Concentrations of IL-1α in pooled samples of culture media conditioned by cholesteatoma tissue for indicated culture periods. (IL-1α level in one of four sets of pooled samples at culture period of 72 to 120 hours could not be assayed.) A) Under indomethacin-free condition. B) Under influence of indomethacin.

IL-1α between its natural and recombinant forms. Existence in the supernatant of cofactors acting with IL-1α has also to be considered and is under investigation in our laboratory, because IL-1-induced bone resorption has been reported to be augmented synergistically by other substances such as tumor necrosis factor.[7] Tumor necrosis factor is reported to be produced by macrophages under several kinds of stimulation such as that by endotoxin.[22] Anti-IL-1α antibody did not affect the bone-resorbing activity in the culture supernatant of cholesteatoma tissue without treatment of indomethacin in which IL-1α was also found. This is probably because the supernatant contained enough PGE2 to induce calcium release from calvaria.

Interleukin-1 is one of the most active substances inducing bone resorption through osteoclast activation,[23] but the localization and/or release of IL-1 is thought to be different between the types of IL-1 as well as between the kinds of IL-1-producing cells; ie, it has been reported that IL-1β is mainly produced and released extracellularly by inflammatory cells such as macrophages/monocytes,[24] but that IL-1α is localized intracellularly or on the surface of such cells.[25] In the present study, only IL-1α was demonstrated in the culture media, whereas IL-1β was not detected by immunoassay as well as bioassay. Therefore, the IL-1α-releasing mechanism in granulation tissue with cholesteatoma remains to be identified, and is now being investigated in our laboratory. Cholesteatoma consists of epithelial keratinocytes of tympanic membrane, so that the most conceivable origin of IL-1α might be keratinocytes,

TABLE 3. ACTIVITY OF INTERLEUKIN-1 AS LYMPHOCYTE-ACTIVATING FACTOR ASSESSED BY ENHANCEMENT OF ^{3}H-THYMIDINE INCORPORATION BY MOUSE THYMOCYTES

Condition of Thymocyte Culture	Radioactivity (cpm) of Murine Thymocytes		
	Without Antibody	With Anti-IL-1α*	With Anti-IL-1β*
Control	14,160± 1,000	—	—
IL-1α 1.25 U/mL	121,960± 5,150	18,430±1,640 (96%↓)†	121,150±7,270 (4%↓)†
0.3 U/mL	68,880± 9,930	15,740±1,030 (97%↓)	50,350±4,910 (34%↓)
Control	5,250± 380	—	—
IL-1β 1.25 U/mL	51,450± 3,240	—	13,670± 780 (82%↓)
0.3 U/mL	30,740± 2,700	—	10,670±1,470 (79%↓)
Control	9,620± 1,770	—	—
IL-1β 2.5 U/mL	125,900± 8,800	141,340±5,760 (0%↓)	—
0.5 U/mL	77,150±11,650	70,950±3,940 (9%↓)	—
Control	3,930± 620	—	—
Cholesteatoma supernatant§			
(I) 24 to 72 h	152,880± 9,700	32,250±3,320 (80%↓)	158,700±9,990 (0%↓)
(II) 0 to 24 h	21,260± 1,570	6,050± 570 (83%↓)	15,860±3,140 (29%↓)
24 to 72 h	58,100± 1,210	8,900±1,720 (89%↓)	45,540±4,490 (23%↓)
72 to 120 h	59,890± 3,550	8,870± 530 (89%↓)	43,390±1,840 (28%↓)

Data represent mean values with SE of triplicate assay.

*Antibody solutions against IL-1α and IL-1β were introduced to thymocyte culture at final dilution ratios of 1:100 and 1:20, respectively.

†Percent of inhibition was calculated after subtraction of control values.

§Several pooled samples of cholesteatoma tissue culture supernatant prepared at indicated time steps with indomethacin were applied to thymocyte culture at dilution ratio of 1:4.

because these cells have been demonstrated to produce IL-1α mainly and release it extracellularly.[26]

Besides bone resorption–inducing activity, IL-1α and IL-1β are reported to have multiple functions,[27] eg, activation of T and B cells, induction of acute-phase reactants in liver, suppression of bone-producing activity of osteoblasts, and stimulation of fibroblasts and other connective tissue cells to proliferate and produce PGE2 and collagenase. Localization or production of collagenase in cholesteatoma and surrounding granulation tissue as well as in eroded bone has been reported by Abramson et al[5] and Moriyama et al.[19] Infiltration and/or proliferation of Leu-3a–positive T lymphocytes and HLA-DR–positive cells beneath the epithelial layer of cholesteatoma tissue were found in our laboratory (unpublished data). Interleukin-1α, which is specifically released from cholesteatoma tissue, may be also concerned with these phenomena and is thought to play a wide variety of roles in the aggravation of inflammation in middle ear cholesteatoma.

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PRIMARY CULTURE OF STRIAL MARGINAL CELLS OF GUINEA PIG COCHLEA: GROWTH, MORPHOLOGIC FEATURES, AND CHARACTERIZATION

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To further investigate the cellular mechanisms involved in the formation of endolymph, primary cultures of marginal cells of guinea pig were established. Minute explants obtained by mechanical dissociation of stria vascularis were plated on collagen type I precoated impermeable substrate in serum-free, hormone-supplemented medium. A confluent layer of epithelial-like cells was obtained within 2 weeks. The cultured cells formed domes, demonstrating that they retain some of their transepithelial properties. Polarization was also suggested by electron microscopic observation of apical microvilli and tight junctions. Immunohistochemical methods revealed that the cultured cells coexpressed cytokeratin and vimentin, demonstrating their epithelial origin, although some degree of dedifferentiation occurred. Thus, a primary culture of marginal cells can be established that may be a suitable model for an in-depth investigation of the function of the marginal cells.

KEY WORDS — cochlea, endolymph, marginal cells, stria vascularis.

INTRODUCTION

Marginal cells (MCs) of the stria vascularis (SV) are known to be directly involved in the formation of endolymph, an intracellular-like fluid that plays a major role in the transduction processes of hearing. Evidence for this involvement has been provided by histologic,[1,2] biochemical,[3,4] and electrophysiologic[5-9] studies, which have demonstrated that these epithelial cells have the capacities required for transepithelial transport. However, the difficulty of access and the structural complexity of the SV have hindered further investigation of the function of this tissue so that the mechanisms of endolymph formation are still not fully understood.

Cell culture is now a well-established tool that may overcome these limitations and thus provide insight into the physiology of the labyrinthine fluids and of the SV. The first attempt to apply this technique to the inner ear was presented by Rarey and Patterson.[10] Using bovine inner ear, they were able to culture MCs from the SV that retained some of their in vivo morphologic characteristics. The aim of the present study was to develop a similar technique with SV from the guinea pig, a more convenient experimental animal. This paper reports 1) the methods used to culture explants of SV from the guinea pig, 2) the growth and morphologic features of cultured cells, and 3) the characterization of their epithelial nature by immunolocalization of keratin filaments in their cytoplasm.

METHODS

Culture Preparation. Young male pigmented guinea pigs weighing 70 to 100 g (Elevage, Ardenay, France) were painlessly decapitated after lethal anesthesia (pentobarbital 100 mg/kg). Bullae were removed and rapidly immersed in ice-cold Eagle's minimal essential medium with Earles's salts (E-MEM). Further dissection was carried out in a laminar flow hood under a dissecting microscope. The cochlea was opened longitudinally and the SV from three basal turns was gently dissected from the spiral ligament with fine forceps and placed in fresh complete culture medium. Minute fragments from this strial tissue were obtained by mechanical dissociation by means of a scalpel and pipette and placed on a 24-well polystyrene culture plate (surface approximately 2 cm², Falcon trademark, Polylabo, Paris, France) previously coated with type I collagen. The culture plate was then placed in an inverted position for 20 to 30 minutes at 37°C to facilitate fragment attachment. The plate was then righted and 400 μL of complete medium was added slowly to prevent mobilization of the recently attached fragment. The next day 600 μL of the medium was added. Cultures were grown at 37°C with maximal humidity in 5% CO_2/95% air and examined daily by inverted microscopy (Wild Leitz). The culture medium was changed twice a week.

Culture Medium. The complete culture medium was red phenol-colored E-MEM in which L-valine was replaced by D-valine to inhibit fibroblast growth. This solution was supplemented with Hepes buffer (20 mmol/L), L-glutamine (2 mmol/L), nonessential amino acids X-100 (10 mL/L), hydrocortisone (0.4 μg/mL), cholera toxin (10^{-10} mol/L), insulin (5 μg/mL), triiodothyronine (2.10^{-9} mol/L), transfer-

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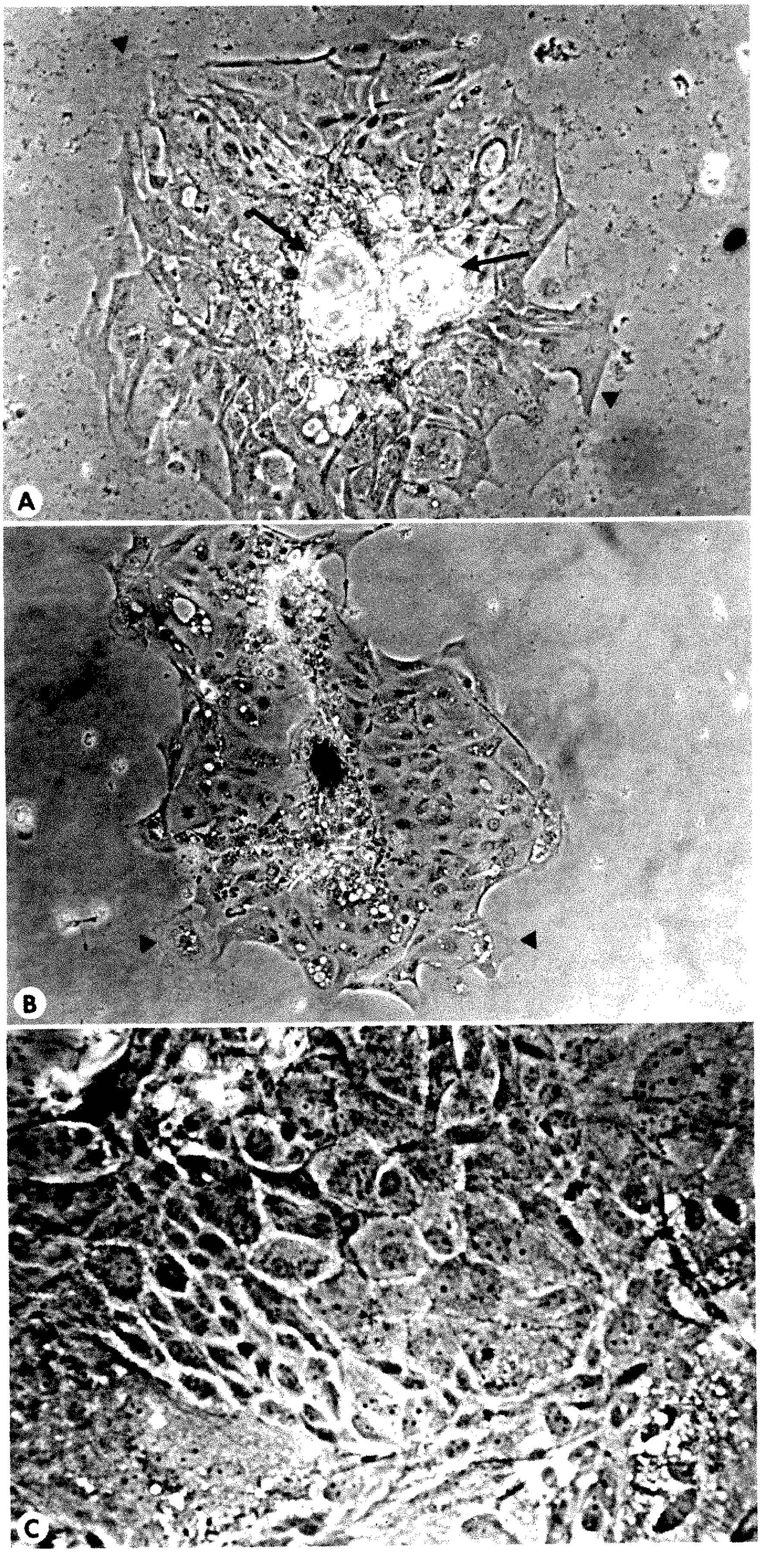

Fig 1. Photomicrographs of explants. A) Day 2 (original x125). Bubble formations (arrows) and cytoplasmic expansions (arrowheads) are clearly seen. B) Day 4 (original x125). Growing cells are visible at periphery of explant. Note foamy appearance of numerous cells (arrowheads). C) Day 14, showing difference in cell density (original x250).

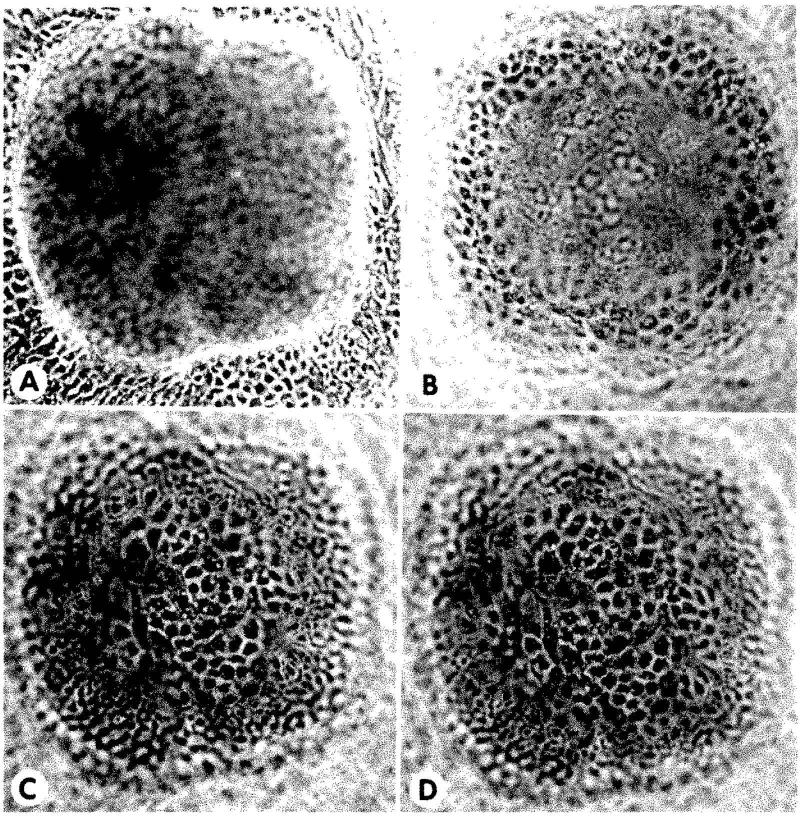

Fig 2. Typical aspects of dome at different depths of focus (A [basal] to D [apical]) on 16th day of culture (original x125). Micropuncture of these domes and analysis of fluid revealed extracellular-type fluid.

rin (5 μg/mL), penicillin (100 U/mL), streptomycin (100 μg/mL), and Fungizone (2.5 μg/mL). Fetal calf serum (10%) was added for plating and replaced at the time of the first change of the medium by epidermal growth factor (10 ng/mL). In order to test the possible influence of Fungizone on the cellular aspect and the dome formation, some culture plates were grown without Fungizone.

Coating of the growth surface of culture plates was made by placing 1 mL of a collagen type I solution (0.2 mg/mL) overnight at 37°C in the incubator. The excess collagen was removed and the growth surface rinsed with 2 mL of E-MEM containing 10% fetal calf serum. Wells were then ready for culture.

Transmission Electron Microscopy. Culture medium was removed and cultures were fixed for 2 hours in 2.5% phosphate-buffered glutaraldehyde (pH 7.4), then rinsed in buffer and postfixed in 1% phosphate-buffered osmium tetroxide (pH 7.4). After dehydration in graded concentrations of ethanol, cultures were embedded in Epon-araldite and cut with a saw. Ultrathin sections (Ultracut, Reichert, Leica, Rueil, France) were made, stained with uranyl acetate and lead citrate, and examined with a Philips 400 electron microscope.

Electrochemical Analysis. The fluid accumulated within domes of epithelial cells was sampled by micropuncture with a De Fonbrune micromanipulator (CIT Alcatel, Paris, France). This was accomplished in 10 domes under stereomicroscopic observation, with a beveled siliconized micropipette that was filled with water-saturated mineral oil (Prolabo, Paris). The tip (outside diameter, 5 μm) was introduced into the dome. A few nanoliters were withdrawn by gentle suction until the dome collapsed, cancelling the negative pressure. The micropipette was rapidly removed and the sample transferred under oil. The concentrations of Na and K were immediately determined in a 0.1-nL aliquot by means of emission ultramicrospectrophotometry.

Antibodies. A primary prediluted mouse monoclonal antibody to pancytokeratin was used. It reacted with cytokeratins of 40, 46, 52, 56, 58, 65, 66, and 67 kd molecular weight. The avidin-biotin-peroxidase complex method was used to visualize the primary antibody.

A primary goat polyclonal antibody to vimentin (1/20) was used. The avidin-biotin–alkaline phosphatase complex was used to visualize the primary antibody to vimentin.

Staining Protocol. The culture plates were fixed with 4% paraformaldehyde for 30 minutes at room temperature, then with methanol for 5 minutes at −20°C. The plates were rinsed 30 minutes in phosphate-buffered saline (PBS) containing 0.25% triton X-100, gelatin (2 g/L), L-lysine (0.1 mol/L), and sodium azide (1 g/L). Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 30 minutes.

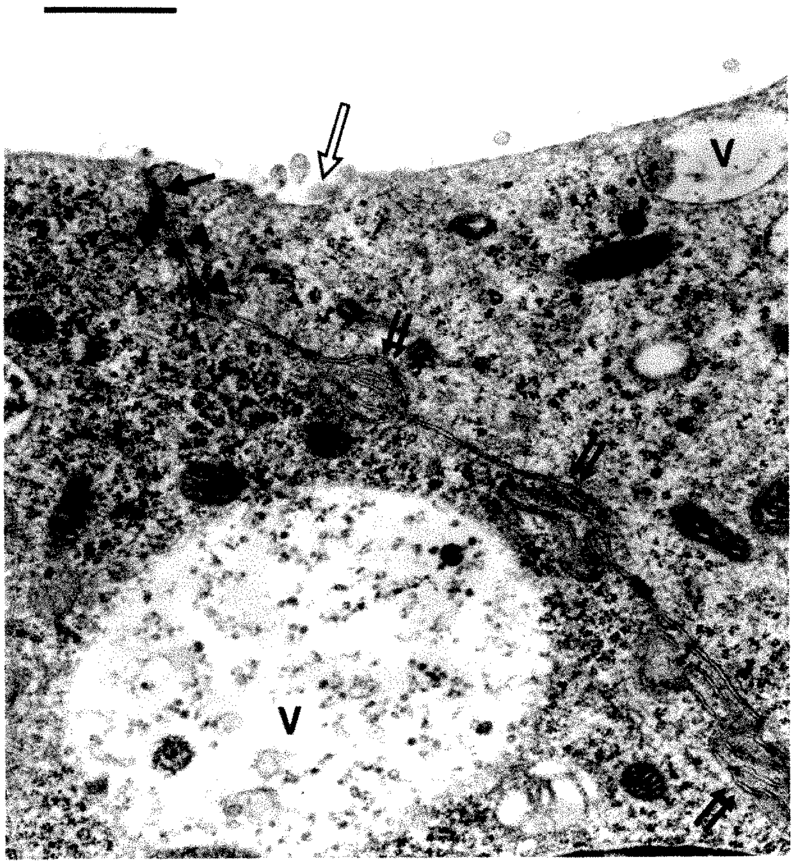

Fig 3. Electron micrograph of cultured cell layer at day 12. Junctional complex is seen between two adjacent cells. Tight junction (solid single arrow) is present at upper part of intercellular space above two desmosomes (arrowheads). Short microvilli are located on apical side (open arrow). Note numerous lateral infoldings of plasma membranes (solid double arrows). Vacuoles (V) and numerous polyribosomes are present in cytoplasm. Bar — 1 μm.

All PBS solutions used for washing and antibody dilutions contained 0.025% triton X-100 and gelatin (2 g/L), except for the one used for antibody to pancytokeratin, which was supplied prediluted. Before each step of the following procedures, three washings of 5 minutes each were performed.

Next, the culture plates were incubated with nonimmune serum of the species from which the secondary antibody was derived to diminish nonspecific reactivity from any secondary antibody. The culture plates were incubated with primary antibodies for 48 hours at 37°C. They were further incubated with secondary biotinylated anti-mouse or anti-goat antibody, then with the avidin-biotin enzyme complex; each incubation step lasted 1 hour at room temperature. The chromogenic substrate for the peroxidase reaction was diaminobenzidine, which produced a brown positive staining. Fast red TR salt with levamisole was used as the chromogenic substrate for the alkaline phosphatase reaction, producing a red positive staining. Levamisole was used to block endogenous alkaline phosphatase.

The staining procedure for keratin and vimentin could be carried out separately on different culture plates or successively on the same culture plate. In the latter case, the culture plate was first incubated with antibody to cytokeratin, then revelation was obtained by the avidin-biotin-peroxidase complex by using diaminobenzidine as the chromogenic substrate. Then after washing with distilled water, the antibody to vimentin was used and revelation was obtained by the avidin-biotin–alkaline phosphatase complex by using fast red TR salt as a chromogenic substrate.

Positive controls such as abdominal guinea pig skin (frozen sections), cultured fibroblast cells isolated from guinea pig middle ear mucosa, and Madin-Darby canine kidney cells in culture were used to prove the activity of the antibodies and the validity of the reaction on cultured cells. To determine the specificity and to avoid false-positive readings, negative controls were run in parallel. In each control case, the cultured plate was first incubated with nonimmune serum of the species from which the secondary antibody was derived to diminish nonspecific reactivity. The primary antibodies were replaced by PBS to reveal background staining.

Reagents. Both E-MEM and nonessential amino acids were obtained from Flow Laboratories (Courbevoie, France); fetal calf serum, Hepes buffer, L-glutamine, and penicillin-streptomycin were from Eurobio (Paris). Cholera toxin, insulin, triiodothyronine, human transferrin, and epidermal growth factor were purchased from Sigma Chemical Company (Coger, Paris). Hydrocortisone was obtained from Laboratoires Roussel (Paris), and

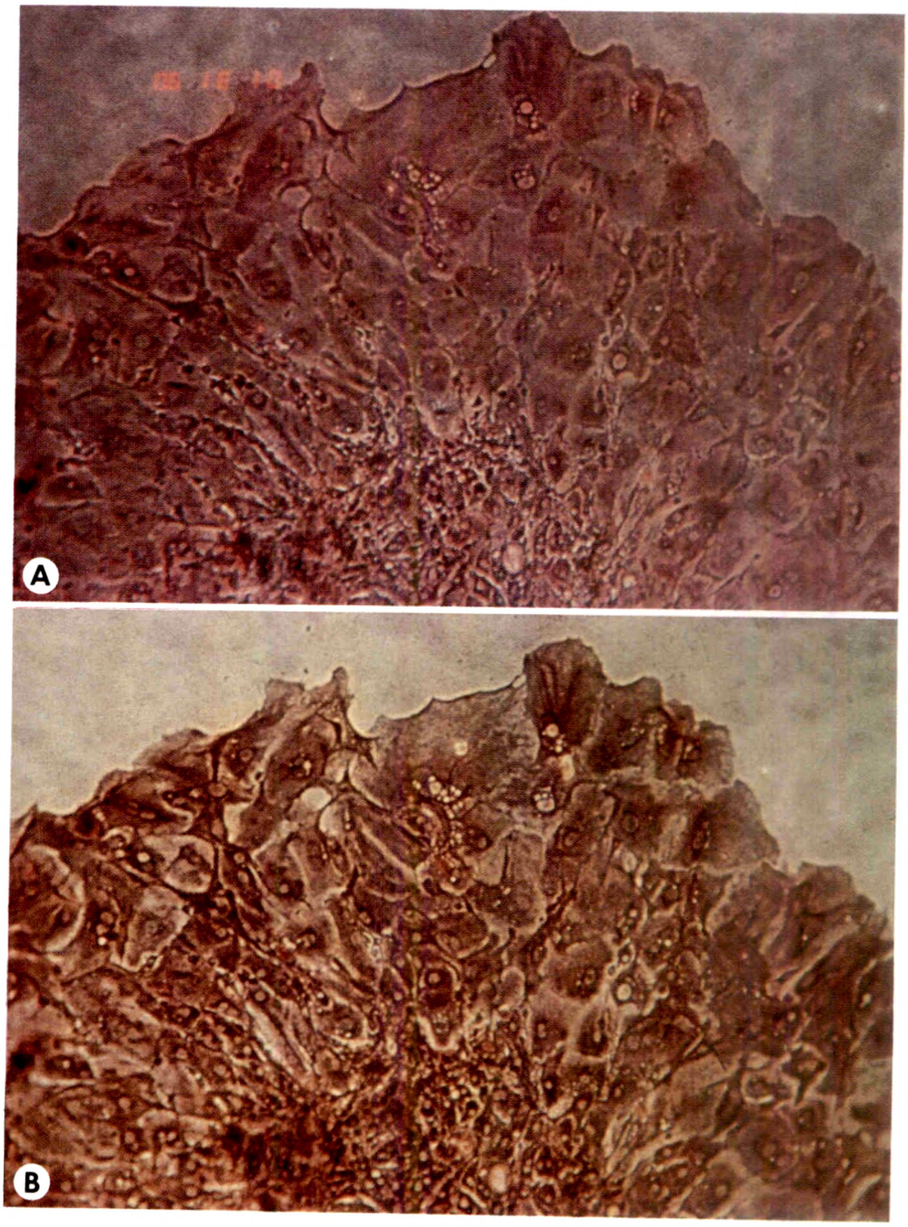

Fig 4. Double immunostaining showing A) red positive vimentin and B) brown positive cytokeratin staining on 10th day of culture (original x125).

collagen type I from rat tail tendon from Institut Jacques Boy (Reims, France). Monoclonal antibody to pancytokeratin was purchased from Amersham (Little Chalfont, Buckinghamshire, England). Polyclonal antibody to vimentin was obtained from Sigma Chemical Company (Coger). The avidin-biotin enzyme (peroxidase and alkaline phosphatase) complexes were obtained from Vector (Biosys, Compiègne, France).

RESULTS

First signs of growth were observed within 24 to 48 hours. They consisted of two salient and almost concomitant features, ie, cytoplasmic expansion and bubble formation (Fig 1A). Cytoplasmic expansion was clearly visible at the periphery of the explant, spreading out centrifugally. Bubble formation was also observed within the explant as highly refrigent spheres lifting up the tissue and rapidly increasing in volume and size. With the involution of the explant, however, these bubbles slowly regressed and finally disappeared. Cells grew centrifugally from the explant (Fig 1B). When several explants were plated on the same well, confluency could be achieved by coalescence of the expanding islands within 2 weeks. Cell density varied within the expanding zones, with the cells in some places tall and tightly packed, in others large and loosely joined (Fig 1C). When confluency was not achieved within 3 weeks, primary cultures seemed to stop growing, suggesting an exhaustion of the cultured tissue. A foamy appearance was observed in numerous cells.

A salient finding of this study was the formation of domes, sometimes visible with the naked eye. They seemed to develop between the surface of the dish and the cell layer. Doming epithelial-like cells covering a cell-free plastic surface were demonstrated by varying the focus of the microscope on the dome (Fig 2). Thus, the surface of these cystic structures appeared to be formed by a monocellular layer. These domes expanded, distended, and collapsed. They were observed with or without Fungizone in the medium. Less frequently, another type of fluid-filled structure was observed at the surface of the cultured cells. It was apparently formed of distended cells lying on a pavement of attached cells, and it developed as a finger or a balloonlike structure. The cells could eventually detach from the surface of the attached cell layer at the time of the medium change. That these fluid-filled structures were only seen when Fungizone was present in the medium suggests that they might represent an adverse effect of this well-known cytotoxic agent.

In some instances, fibroblastlike cells also developed at the periphery of the explant. They proliferated rapidly, either at random with no tendency to become confluent, or forming strands that in a few cases encircled areas of epithelial-like cells. In these surrounded zones, the epithelial-like cells became tightly packed, compact, and polygonal. Growth of fibroblastlike cells occasionally hindered the expansion of epithelial colonies. As already described by Rarey and Patterson,[10] they could be removed by enzymatic digestion. The epithelial layer was then incubated again in growth medium. Occasionally, another type of cell was clearly identified that presented some of the usual characteristics of nerve cells in that they had a cell body and dendritelike expansions.

Electron microscopy of the cultured epithelial cells revealed the morphologic characteristics of MCs present in vivo, ie, apical microvilli extending upward into the medium and occluding junctions and desmosomes between adjacent cells at the apicolateral membrane border (Fig 3). Numerous mitochondria, ribosomes, and rough endoplasmic reticulum were observed in the cytoplasm.

The fluid sampled from domes appeared transparent under the microscope in contrast with the red phenol–colored medium. The Na and K concentrations were 132±11.7 mmol/L and 3.7±1.2 mmol/L (mean±SE, n = 10), respectively.

Cytokeratin and vimentin staining occurred in cytoplasm of MCs, whereas fibroblastlike cells gave a negative cytokeratin staining but a positive vimentin staining. In the double staining procedure, the monolayer showed first a brown cytokeratin staining and then a red vimentin staining (Fig 4). Keratinocytes of the abdominal skin of the guinea pig and cultured Madin-Darby canine kidney cells showed a positive cytokeratin staining. Cultured fibroblasts, which are of mesenchymal origin, showed a positive staining to vimentin. Negative controls gave no staining.

DISCUSSION

Cell culture offers numerous advantages for the study of cell biology, because 1) it provides homogeneous material that minimizes variability among samples in an experiment, 2) it allows the study of a given type of cell under defined conditions, and 3) it facilitates the investigation of the transport mechanisms in epithelia, especially of the systems located on the apical membrane of the cultured cells.[11] Because of the numerous difficulties encountered in investigating endolymph secretion, the above-mentioned advantages prompted us to apply this technique to the MCs of the SV, which are known to be directly involved in the secretion of this fluid.

The present study reports the growth, morphologic features, and characterization of MCs in culture. It differs from the one presented by Rarey and Patterson[10] in that 1) the guinea pig was used, because it is a more convenient experimental animal than the cow, and 2) the culture grew from explants obtained by mechanical disruption of the SV and not from enzymatically dissociated MCs, because the very small amount of fresh tissue that could be harvested from the guinea pig cochlea made it difficult to isolate a sufficient quantity of these cells. Histologic controls of these explants showed that in most cases they consisted of marginal, intermediate, and endothelial cells. Basal cells were rarely seen. Thus, the explant was not homogeneous. Nevertheless, it was found that under our experimental conditions, contamination of the culture by mesenchymal cells was rare.

Confluency of growing islands was achieved by plating several minute explants in the same well. The cultured cells exhibited light and electron microscopic features characteristic of an epithelium. The morphologic appearance under inverted microscopy was highly suggestive of their epithelial nature. Of interest is the observation that the cell density and, moreover, the polygonal aspects of the cells were markedly enhanced when confluency was achieved within the culture plate or when, infrequently, strands of fibroblasts surrounded the growing area. This finding is in agreement with the well-established fact that heterotypic cell interaction is required for the complete differentiation of most tissues.[12] Electron microscopic study clearly identified apical and lateral specializations, with apical microvilli, tight junctions, and mitochondria. These observations strongly suggest that a polarization developed and that the cultured tissue attempted to become functional, as it has been already demonstrated in other transporting epithelial cells grown in culture.[13,14]

A striking phenomenon observed in the present study was the development of bubblelike formations within the explant as well as in the epithelial-like cells that grew beyond the margin of the explant. This foamy appearance observed under an inverted microscope (Fig 1B) and evidenced by the numerous intracytoplasmic vacuoles clearly seen on electron microscopy (Fig 3) has been already described in other ion-transporting cells grown with[10,15] or without[16,17] Fungizone. It suggests that a mechanism involving an intracellular accumulation of fluid could be operative. Another salient observation was the formation of domes. These domes represent localized regions of fluid accumulation between a layer of cultured cells and the surface on which they grow. Dome development required three conditions: 1) an impermeable substrate,[18] 2) an effective paracellular permeability barrier provided by tight junctions,[19] and 3) a net unidirectional ion transport provided by polarized cells.[20] Compelling evidence suggests that Na is the ion transported and secondarily responsible for water movement.[21] Thus, the presence of fluid-filled, hemicyst formations demonstrates that the cultured epithelial-like cells retained in vitro some of their transepithelial transport properties.[22]

Because MCs are the only strial cells of known epithelial origin, characterization of the intermediate filaments located in the cytoplasm of the cultured cells was performed by means of immunohistochemistry. These proteins are expressed in a tissue- and cell-type–specific manner. Thus, antibodies to cytokeratin and to vimentin were used to distinguish cells of epithelial and mesenchymal origin. Expression of intermediate filaments in SV has been investigated by a few researchers. As a rule, MCs expressed cytokeratins while intermediate and basal cells expressed vimentin in normal[23] or fetal[24] mice, dancer mouse mutant,[25] gerbil, cat,[26] guinea pig, and rat.[27]

In the present study, the cultured cells coexpressed cytokeratin and vimentin. Coexpression of different immediate filament subunits is not a common phenomenon but has been observed in a number of established mammalian cell lines of epithelial origin.[28,29] It may be accounted for either by extrinsic factors such as modifications in the culture medium or in the composition of the substrate on which cells were grown,[30] or by loss of cell-to-cell contact in cultured epithelial cells,[31] which could modulate the expression of immediate filaments. Still, the prominent feature remains that the cultured cells expressed cytokeratins, demonstrating their epithelial nature.

In conclusion, the present study demonstrates that a primary culture of strial MCs may be established on impermeable substrate. Light and electron microscopic features, formation of domes, and cytokeratin-positive immunostaining strongly suggest that our cultured epithelial-like cells do correspond to MCs. Preliminary results of electrophysiologic studies using the patch clamp technique suggest that this type of culture may be a suitable model for an in-depth investigation of the function of MCs.

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FURTHER MORPHOLOGIC STUDIES ON COLLAGEN INJECTED INTO CANINE VOCAL FOLDS

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We compared the morphologic fate of three types of soluble bovine collagen injected into vocal folds of dogs: 1) Zyderm collagen implant containing 35 mg/mL of collagen (ZCI I), 2) Zyderm collagen implant containing 65 mg/mL of collagen (ZCI II), and 3) GAX collagen containing 35 mg/mL of collagen minimally cross-linked with 0.0075% glutaraldehyde. Light and electron microscopy showed good host tolerance and a long-term persistence of the three types of collagen at the site of injection. The collagen implants were progressively colonized by fibroblasts and capillaries accompanied by host collagen deposition. No inflammatory process or foreign body reaction occurred, in contrast to the typical host reaction to injected Teflon. The colonization of GAX collagen was slower than for ZCI I and ZCI II. No other significant difference was evident among the three types of injectable collagen. All three appear to be very suitable materials for laryngeal implants; moreover, they seem to best meet accepted requirements for a good implant: they are easy to inject, they are well tolerated, and they persist for a long time. We prefer GAX collagen for clinical use because of its lower reactivity and higher stability, and because overinjection is not necessary (GAX shrinks less than ZCI I and ZCI II).

KEY WORDS — collagen, glottic insufficiency, larynx, morphology.

INTRODUCTION

In 1962, Arnold[1] used injectable Teflon to rehabilitate paralyzed vocal folds and thus reintroduced Bruning's technique into clinical medicine. This technique is still widely practiced,[2-7] but because the use of Teflon is not trouble-free,[8-12] the search for an alternative material seems warranted. Injectable collagen has been proposed for rehabilitating the vocal folds and larynx,[13-15] and more than 250 patients[16-18] have been treated since the first experimental work by Ford[19] and us.[20]

Histologic examinations of human laryngeal specimens[21,22] have confirmed that injected collagen is well tolerated by the host and is well integrated into the vocal fold lamina propria. One advantage of using collagen is that it affords a fine point of delivery and thus a precise placement of the material. Indeed, collagen can be placed in superficial planes in which Teflon would not be tolerated.

Injection of collagen into the superficial vocal fold does not create a stiffness imbalance between the vocal folds. The persistence of the mucosal traveling wave is observed under videostroboscopic examination.[15,16,23]

We studied different types of injectable collagen and here compare the medium-term fate of three collagens injected into canine vocal folds: Zyderm collagen implant containing 35 mg/mL of collagen (ZCI I); Zyderm collagen implant about 85% more concentrated, containing 65 mg/mL of collagen (ZCI II); and glutaraldehyde cross-linked (GAX) collagen.

Zyderm collagen implant is a highly purified suspension of a bovine dermal collagen in a buffered physiologic saline solution containing 0.3% lidocaine. The product contains at least 95% type I collagen; type III collagen makes up the remaining 1% to 5%. The nonhelicoid amino-terminal and carboxy-terminal segments (telopeptides) are the most antigenic sites of the collagen molecules. The telopeptides are selectively hydrolyzed during the preparation of injectable collagen, and thus mark-

EXPERIMENTAL DATA

Dog	*Injected Material*	*Vocal Fold Injected*	*Quantity (mL)*	*Sample-Taking Mode*	*Time*
1	ZCI I	L	0.75	Vocal fold resection	3 wk
2	ZCI I	L	0.75	Vocal fold resection	1 mo
3	ZCI I	L	0.75	Vocal fold resection	3 mo
				Laryngeal resection	6 mo
4	Teflon	L	0.75	Vocal fold resection	4 mo
	ZCI I	R	0.75		
5	ZCI II	L	0.75	Vocal fold resection	4 mo
	ZCI I	R	0.75		
6	ZCI II	L	0.75	Vocal fold resection	6 mo
	ZCI II	R	0.75		18 mo
7	Teflon	L	0.50	Laryngeal resection	3 mo
	ZCI II	L	0.25		
	GAX	R	0.75		
8	GAX	L	0.75	Biopsy 1	3 wk
				Biopsy 2	3 mo
9	GAX	L	0.75	Biopsy 1	2 mo
				Biopsy 2	4 mo

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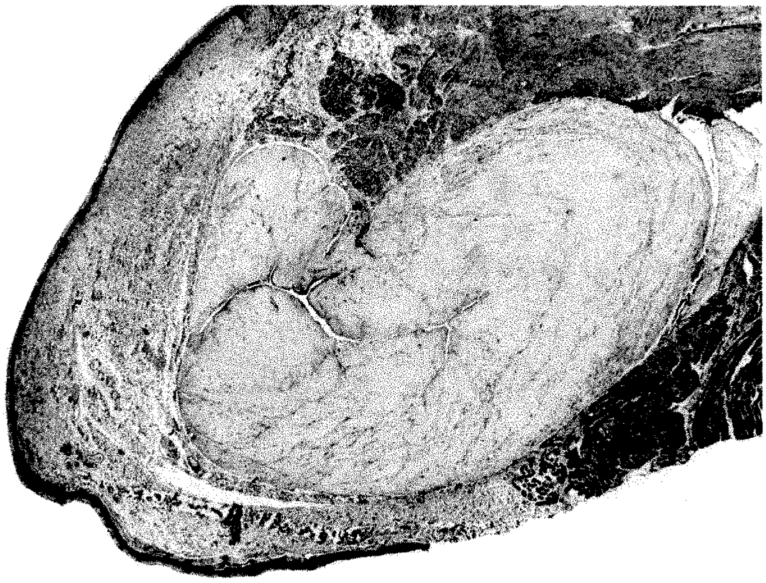

Fig 1. (Dog 1) Large nodule of ZCI I collagen 3 weeks after injection (hemalum, eosin, saffron, original x20). Notice smooth borders of nodule, injected in deep portion of lamina propria and protruding into vocal muscle without destroying it. Irregular clefts are present in collagen bulk, and peripheral colonization of injected collagen is obvious.

edly reduce antigenicity. Hydrolysis reduces both intermolecular and intramolecular cross-links while preserving the basic tropocollagen molecule. Sterilization is improved if the material is progressively passed through ultrafine filters.[24]

GAX collagen is prepared first as ZCI collagen, and then minimally cross-linked with 0.0075% glutaraldehyde before being suspended in a phosphate-buffered saline solution at a concentration of 35 mg/mL. The cross-linking provides two advantages[25,26]: 1) the collagen fibrils are much more collagenase-proof, and 2) there is no volume reduction. (With ZCI collagen, by contrast, the elimination of 40% to 60% of the saline after injection consolidates the collagen.) Furthermore, GAX collagen seems to result in lower hypersensitivity: fewer than 1% of reported cases, against 3% for ZCI collagen.[27]

MATERIALS AND METHODS

Materials injected into canine vocal folds included ZCI I and ZCI II collagens (Zyderm, Collagen Corp, Palo Alto, Calif) and GAX collagen (Phonagel, Collagen Corp). We also injected Teflon glycerin paste (Polytef, Mentor Division, Godman & Shurtleff, Randolph, Mass) to compare inflammatory reactions. Teflon was injected with a Bruning's syringe.[1-7] The first ZCI and GAX collagen injections were made through an ordinary laryngeal needle used for vocal fold hydrotomy,[14] but since the end of 1985 we have instead used a device that was specially designed for vocal fold and laryngeal injections.[16,17] This device consists of a handpiece that secures a tuberculin syringe to a long, fine tube that terminates in a 27-gauge disposable needle. Collagen is fluid enough to pass easily through the tube and needle without developing back pressure and run-on after injection. That the capacity of the tube is only 0.13 mL ensures only a minimal waste of material.

The experimental animals were nine adult mongrel dogs with a mean weight of 20 kg. Anesthesia was routinely induced with fluonicone-fentanyl (Hypnorm) and maintained with ketamine (Ketalar). The injections and the controls for vocal fold resection and biopsy were visualized by direct laryngoscopy. The same volume (0.75 mL) was injected regardless of the substance used. The puncture was usually made in front of the arytenoid vocal pro-

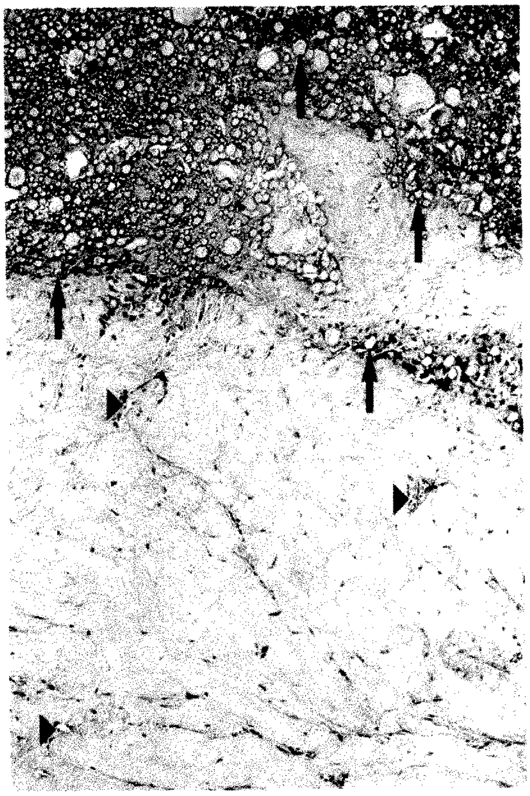

Fig 2. (Dog 7) Left vocal fold (hemalum, eosin, saffron, original x125). Teflon and ZCI II collagen injected together. Intense macrophagic reaction is present against Teflon particles (arrows), whereas ZCI II collagen is colonized by fibroblasts and capillaries (arrowheads).

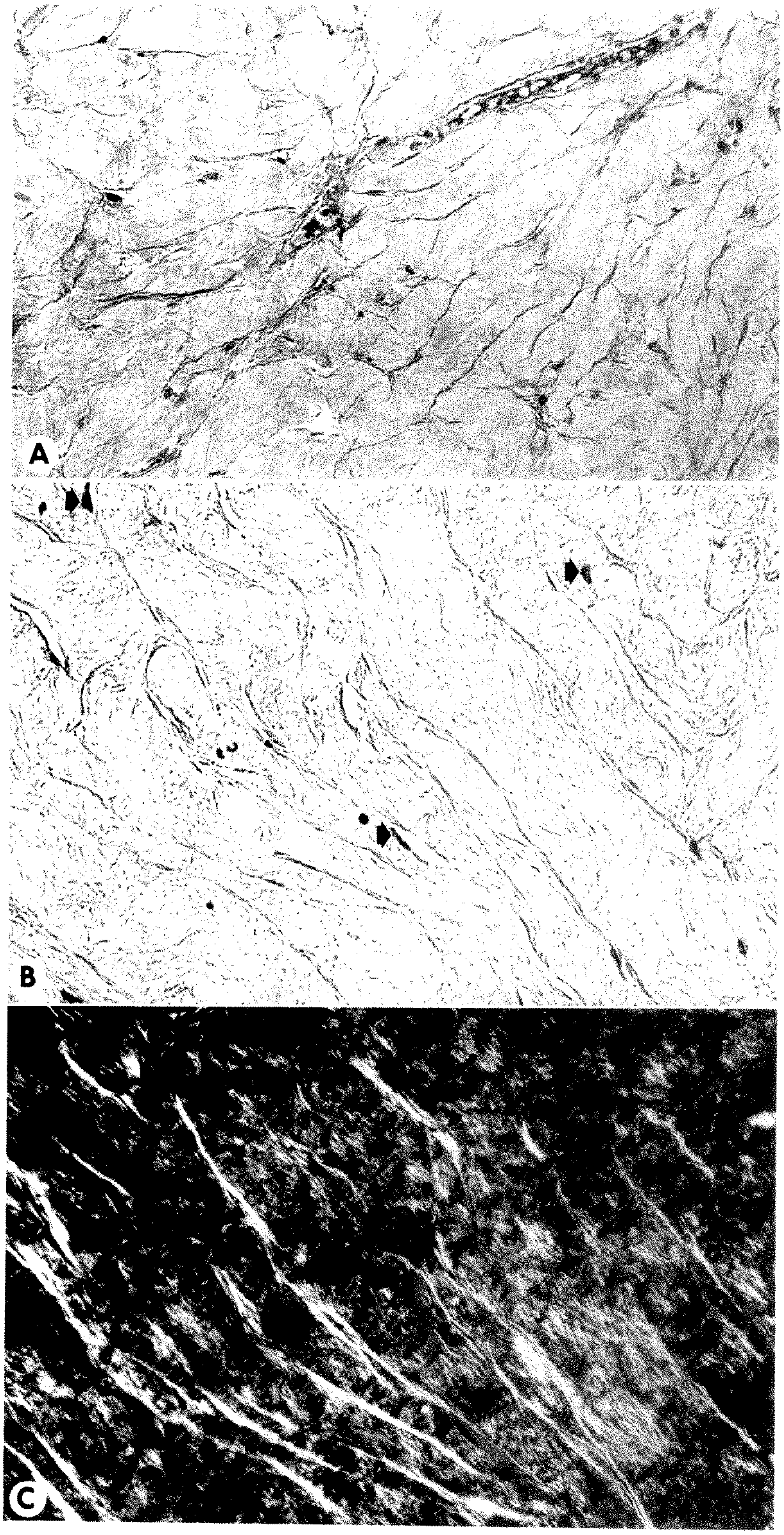

Fig 3. (Dog 6) A) Left vocal fold (hemalum, eosin, saffron, original x300). ZCI II collagen is colonized by fibroblasts and one capillary. Newly formed host collagen is deposited along tracks of colonizing cells and appears as dark lines. B,C) Right vocal fold (hemalum, eosin, saffron, original x500). B) Fibroblasts seem to colonize ZCI II collagen along slits outlined by dark fibers and newly formed host collagen. C) Same field as in B, examined with polarized light. Host collagen fibers are brightly birefringent, whereas ZCI collagen is moderately birefringent. Arrows point to same cells in both B and C.

cess, and the injection was limited as much as possible to the vocal fold submucosa. Samples were routinely fixed in formalin or Bouin's solution and embedded in paraffin for light microscopy. Five-micron sections were stained with hemalum, eosin, and saffron, periodic acid–Schiff stain, Alcian blue at pH 2.5, Masson's trichrome, and reticulin preparation according to van Gieson's method.

For dogs 8 and 9, samples were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, embedded in Epon 812, and cut in a Reichert Jung Ultracut E ultratome. One-micron-thick sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 109 electron microscope (Zeiss, Oberkochen, Germany).

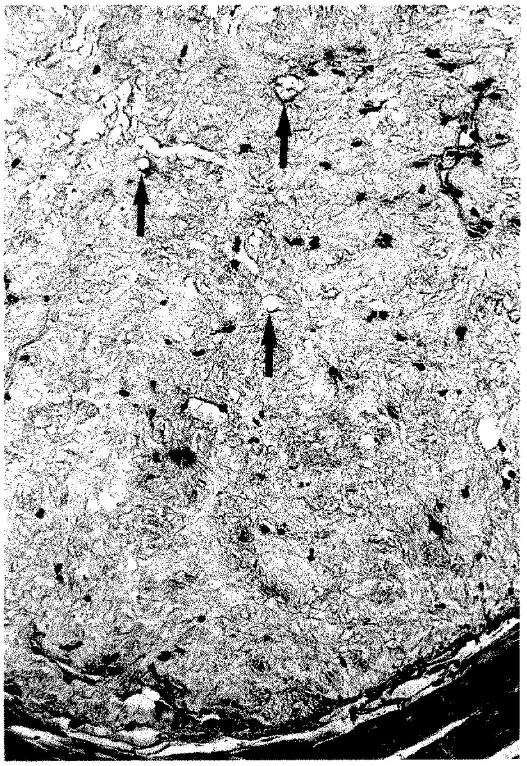

Fig 4. (Dog 9) Biopsy 2 (Masson's trichrome, original x300). GAX collagen looks more reticulated than ZCI collagen. Many cells colonizing GAX collagen 4 months after injection appear vacuolated (arrows). Notice well-preserved muscle fibers in close contact with injected collagen.

The details of the experiments are shown in the Table. Uninjected ZCI and GAX collagens were examined by light microscopy; uninjected GAX collagen was also examined by electron microscopy.

RESULTS

In all animals, injected collagen formed nodules of various shapes and sizes in the lamina propria or the vocalis muscle (Fig 1). There was no necrosis of the surrounding tissues, and no macrophagic activity against the injected collagen; this was a marked contrast to the impressive macrophagic resorption that resulted from injected Teflon (Fig 2). Scarce small aggregates of lymphocytes and plasma cells at the edge of some nodules were the only evidence of inflammation. The host native collagen, ZCI I collagen, and GAX collagen all had a different morphologic appearance.

At low magnification (Fig 1), the nodules of ZCI collagen looked more homogeneous and hypocellular than the fasciculated host collagen, but slits were evident in the nodules, and cells invaded the injected collagen.

Colonization was obvious just 3 weeks after injection, and was most prominent at the periphery of the nodule. Subsequently there was no significant increase in the number of colonizing cells, and some central areas remained almost acellular 4 months after the injection.

At higher magnification (Fig 3), the scattered colonizing cells appeared to be mostly fibroblasts, but there were some lymphocytes. A few capillaries were apparent inside the injected collagen, sometimes surrounded by a delicate rim of Alcian blue–stained material or by a few reticulinic fibers. The colonization seemed to mainly proceed along lines that probably corresponded to small slits in the collagen bulk. Collagen fibers outlined the tracks of the colonizing cells. These fibers were long, dark, and small; sometimes they were aggregated into thin bundles that dissociated the collagen bulk into small strands of very small thin fibers that were more disorderly in arrangement. These thin fibers showed a moderate birefringence with polarization, identical to that of uninjected ZCI collagen. The elongated dark fibers, by contrast, showed more intense birefringence, as did the surrounding host collagen. There was no apparent morphologic difference between ZCI I and ZCI II collagens, and the morphologic appearance of injected collagen did not change over the 18 months of observation.

GAX collagen formed more irregular nodules with larger and sometimes more numerous slits than ZCI collagen, and therefore looked less cohesive. Colonization was less rapid and less intense, so that GAX collagen nodules appeared almost acellular in large central areas. Only rarely were colonizing cells seen at the edge of the nodules in samples taken 3 weeks after injection; no colonization was evident in the small irregular nodules taken 2 months after injection. Peripheral colonization was more pronounced 3 and 4 months after injection (Fig 4). Some cells found inside the GAX collagen appeared to be vacuolated. The colonizing cells were accompanied by very rare, small collagen fibers that showed an intense birefringence on polarization (Fig 5); the main part of the collagen showed very weak birefringence, identical to that of uninjected GAX collagen. GAX collagen was more faintly stained by saffron, Masson's trichrome, and van Gieson's stains than host and ZCI collagens, and it appeared to be made up of a very delicate network of short microfibers. This histologic appearance did not change with time.

The electron microscopic study was done on uninjected GAX collagen, and on GAX collagen 3 weeks and 2 months after being injected. The ultrastructure of GAX collagen appeared as a meshwork of small dots and short fibrils that outlined irregular processes of amorphous material. Three weeks after the injection, fibrillar material was evident at the edge of the GAX collagen mass but not around the cells that invaded the collagen (Fig 6). The cells that limited or invaded the GAX collagen often appeared as large cytoplasmic clear zones of vacuolar degeneration and irregular projections into the

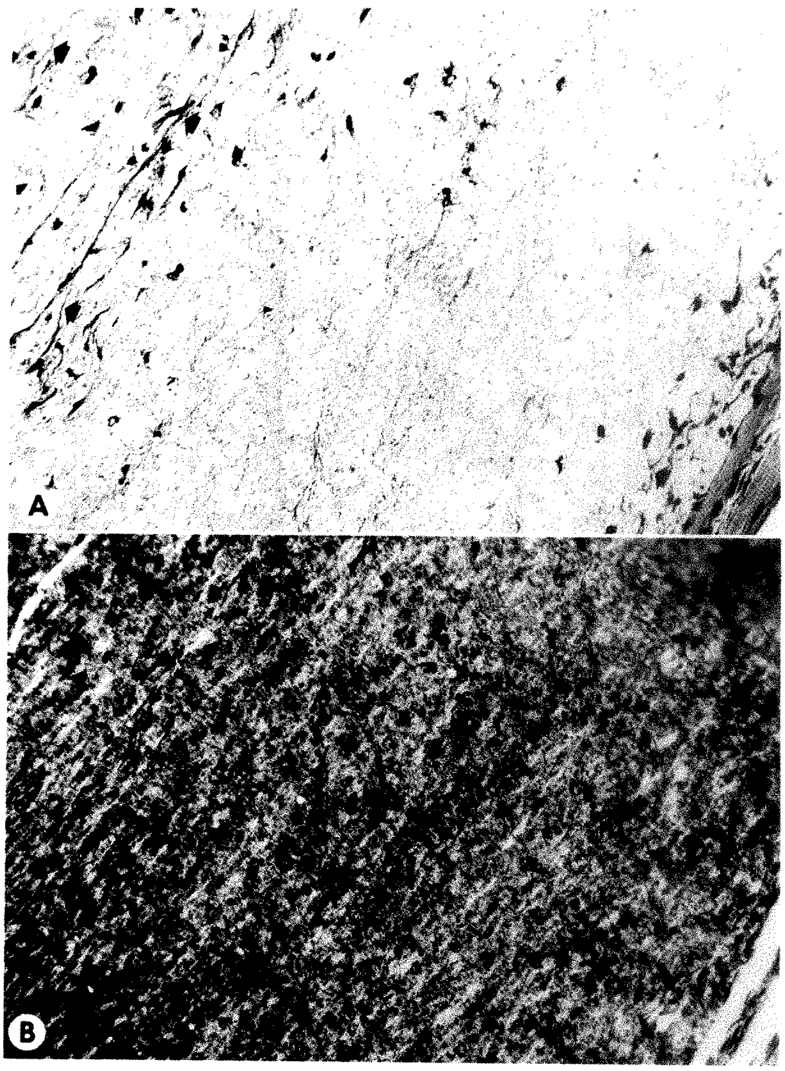

Fig 5. (Dog 7) Right vocal fold (hemalum, eosin, saffron, original x300). A) Colonization of GAX collagen is still focal 3 months after injection, and some cells appear vacuolated. Rare thin fibers of host collagen are deposited along colonizing cells (arrows). B) Same field, with same cells highlighted by arrows, examined with polarized light. GAX collagen is weakly birefringent, whereas host collagen fibers are brightly birefringent, as are muscle fibers crossing lower right corner of picture.

GAX collagen matrix; some of them encircled small nodules of dense, granular material (Fig 6B). Fibers of collagen, clearly different from GAX collagen, were found to limit and invade the GAX collagen 2 months after the injection. The fibrillar material deposited at the edge of the GAX collagen formed dense amorphous bodies and seemed to merge with collagen fibrils (Fig 7).

DISCUSSION

Injected bovine collagen persists and is morphologically identifiable in vocal folds of dogs for as long as 18 months. It forms nodules that push aside the adjacent host structures, and causes neither necrosis nor inflammation. The injected collagen is colonized by fibroblasts and capillaries, and newly formed host collagen is deposited along the colonizing structures. These findings confirm the results of previous studies by Ford et al[13,19] and us.[14,20] There are no morphologic differences between ZCI I and ZCI II collagens, but GAX collagen looks less homogeneous and is less rapidly and less densely colonized than either type of ZCI collagen. Indeed, GAX collagen implants remain almost acellular for the first 2 months after injection.

Differences in the homogeneity of ZCI and GAX collagens are due to differences between the preparations of these substances before they are injected. The ZCI collagen's being solubilized in saline solution accounts for 40% to 60% of the injected volume. After ZCI collagen is injected into the vocal fold, the saline solution is resorbed and the collagen shrinks to form a homogeneous mass. By contrast, GAX collagen, cross-linked with glutaraldehyde, does not eliminate any saline solution. There is therefore no postinjection shrinkage; this factor may account for the less compact appearance of GAX collagen. GAX collagen was more rapidly colonized in Ford's study[19] than in ours. We agree with Ford that colonization seems to proceed along slits in the collagen mass; however, we did not see a more rapid or more dense cellular invasion with GAX than with ZCI collagen, despite the greater number of fissures we saw inside GAX collagen.

The cellular colonization of injected collagen is, of course, a host reaction that likely varies from one dog to another. Ford suggested that the structure,

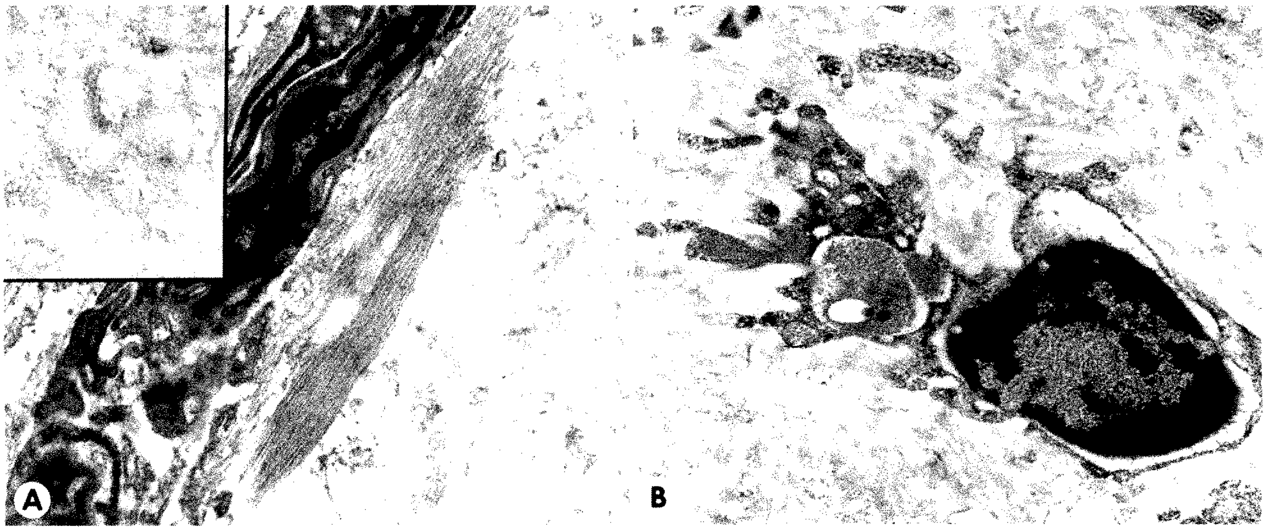

Fig 6. (Dog 8) Biopsy 1 (original x50,000). A) Fine parallel fibrils are deposited at edge of GAX collagen mass. Inset) Amorphous appearance of GAX collagen. B) Cell inside GAX collagen injected for 3 weeks showing cytoplasmic vacuolar degeneration and irregular processes, some of them encircling dense granular material. No fibrils of newly formed collagen are seen around cell.

function, and fate of injected collagen implants may be more attributable to the site of placement than to the biochemical composition of the implant.[19] If collagen is injected into the muscle of mobile vocal folds, muscular activity may disperse the collagen before colonization occurs in the host tissue. And if elastic fibers can break because of muscular activity, so too can collagen fibers. Broken collagen fibers are more likely to be resorbed than unbroken fibers. By contrast, the injection of collagen into the lamina propria of the vocal fold causes a progressive colonization of the collagen by host cells, and there is little breakdown of the implant. Because collagen is a constituent of the normal extracellular matrix of the lamina propria, the implant resembles the tissues in which it is placed. The absence of substantial lymphatic drainage in the superficial lamina propria helps the implanted collagen to persist. Overcorrection during injection is not necessary: the collagen does not disperse and thus the volume needed to reconstitute the shape of the vocal fold (after paralytic atrophy or a surgical defect) is less than the volume needed when a filler substance is injected into the muscle.[13-17] The discrepancy in GAX colonization between Ford's results and ours could therefore be due to a different localization of the injected collagen.

Another explanation for this discrepancy could be the glutaraldehyde cross-linking of collagen. The colonizing cells seen inside GAX collagen masses showed signs of alteration that we attribute to the presence of a small amount of glutaraldehyde. Indeed, the presence of this very efficient cross-linking

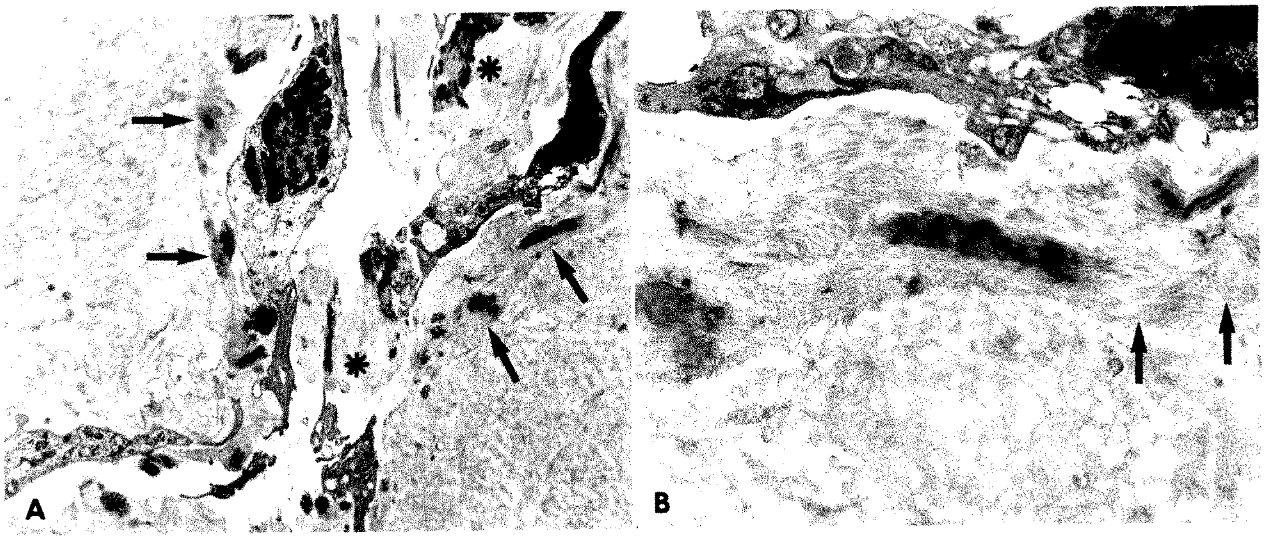

Fig 7. (Dog 9) Biopsy 1. A) Cells in slit in GAX collagen, accompanied by collagen fibers (asterisks) deposited in center of slit and fibrillar material forming condensation at edge of GAX collagen (arrows; original x7,000). B) Higher magnification of part of A (original x25,000). Fibrillar material deposited at contact of GAX collagen forms dense bodies and seems to merge with collagen fibrils (arrows).

agent could impair cellular function and account for a lower rate of collagen deposition by these colonizing fibroblasts; this could be one reason for the delayed colonization and collagen deposition we noted during the first 3 months after GAX collagen injection. However, Ford[19] saw a very different GAX colonization in implants more than 1 year after injection. This difference could be related to the removal of the small amount of glutaraldehyde by the few cells and capillaries that invaded the GAX collagen during the first months after the injection and thus allowed a more rapid and florid cellular colonization along the numerous slits scattered in the collagen mass. Regardless of the cause of the delay and the intensity of colonization, the end result with respect to transformation into living tissue is the same for both types of injectable collagen.

In contrast to the typical host response to injected Teflon, there was no sign of a foreign-body reaction, inflammation, or granuloma formation in any of the collagen-injected specimens. Collagen may not be the ideal bioimplant for vocal fold augmentation, but so far it comes closest to meeting Arnold's[1] criteria for an ideal implant: it is easily injected, is long-lasting, and is well tolerated. Both ZCI and GAX collagens are integrated into the vocal ligament, but we prefer GAX for the reasons stated. In addition, GAX collagen is probably more insensitive to collagenase, and because its antigenicity is less than that of ZCI there is a less than 1% chance of provoking any immunologic response. Overall, there is less risk of inflammation than with any other injectable filler substance, and the lower antigenicity further reduces the possibility of morbid edema, dyspnea, and airway obstruction.

CONCLUSION

This study confirms the views expressed by Ford and us about the fate of collagen more than 1 year after it is injected into canine vocal folds. It does persist, and it appears to be progressively replaced by host collagen during the periods studied. Persistence could be related to the site of injection; the optimal site is in the plane of the vocal ligament. There is no evidence of foreign body reaction or other inflammation. The hypothesis that injectable collagen is a suitable laryngeal implant is supported, in that collagen appears to meet the basic requirements of injectability, host tolerance, and long-term persistence. Our observations also suggest that injectable collagen best fulfills the requisites for an ideal bioimplant: it is porous, it permits the ingrowth of host cells, and, over time, it is replaced by host tissues. GAX collagen seems to us more appropriate than ZCI for vocal fold injection because of its lower host reactivity and higher stability, and because it shrinks little and thus eliminates the need for overinjection.

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COCHLEAR BONE EROSION: EFFECTS ON COCHLEAR HAIR CELLS

A SCANNING ELECTRON MICROSCOPY STUDY

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Cochleae from gerbils with normal middle ears were compared with cochleae from gerbils with experimentally induced cholesteatomas that were in contact with the cochlear wall (stage III cholesteatomas). Cochleae from gerbils with stage III cholesteatomas were further divided into two groups: one without erosion of cochlear bone, and one with cholesteatoma-induced bone erosion but without cochlear fistulae. The cochleae with bone erosion showed significant loss of outer hair cells in the middle and apical turns, but not in the basal turn. The cochleae with stage III cholesteatomas but without bone erosion did not differ from normal controls. These results suggest that an ototoxic agent, involved in the process of bone erosion, acts through the bony cochlear wall.

KEY WORDS — aging, cholesteatoma, gerbil, otitis.

INTRODUCTION

In middle ear cholesteatoma, sensorineural losses may occur as a result of chronic otitis media. Inflammatory products from the middle ear may traverse the round window membrane and damage cochlear tissues.[1] A study in older gerbils (> 1 year) suggests that damage to cochlear hair cells may also directly result from biochemical events occurring during cholesteatoma-induced resorption of cochlear bone.[2] In older gerbils with either experimentally induced cholesteatomas or spontaneous cholesteatomas, cochlear hair cell loss increases with increasing size of the cholesteatomas.[2] However, in these older animals, cochlear hair cell loss can be found even in the absence of an ongoing middle ear infection or of a cochlear fistula.[2] The loss of hair cells was most pronounced in those cochleae that were in direct contact with the cholesteatomas. The loss of hair cells was most pronounced in that portion of the organ of Corti adjacent to the area of greatest contact with the cholesteatoma: the outer portion of the middle cochlear turn.

The purposes of this study were to determine if loss of cochlear hair cells occurs early in the development of a cholesteatoma, and if the loss of cochlear hair cells is related to resorption of cochlear bone.

METHOD AND MATERIALS

The subjects for this study were Mongolian gerbils, *Meriones unguiculatus*, the offspring of breeding stock obtained from Tumblebrook Farms and raised in our vivarium. All animals had free access to water and pelleted laboratory chow (Purina) and were maintained on a 12-hour light-dark cycle. Cholesteatomas were induced by occlusive ligation of the external auditory canal (EAC) on postnatal day 12.[3] Under methoxyflurane anesthesia, 3-0 silk ligatures were introduced through a postauricular incision, passed through a subcutaneous tunnel around the EAC that was formed by blunt dissection, and tightly tied. The incision was then closed with 4-0 silk sutures. The animals were placed in a warmed cage (32°C) for recovery and returned to the colony.

After periods of 3 to 4, 7 to 8, or 10 to 12 months, the animals were painlessly killed by injection with excess sodium pentobarbital (>120 mg/kg) and transcardially perfused with a solution of 2% paraformaldehyde and 2% glutaraldehyde in 0.08 mol/L cacodylate buffered to pH 7.35. The middle ears were then examined through openings in the bullae. For the principal portion of this study, cochleae were taken from normal control ears and from ears with stage III cholesteatomas.[4] Stage III cholesteatomas were those in which the cholesteatoma arising from the tympanic membrane was in direct contact with the bony cochlear wall. Three cochleae with earlier-stage cholesteatomas, not in contact with cochleae, were studied for comparative purposes. Suitable cochleae were fixed further with the same mixed aldehyde solutions by gentle perfusion through the oval window and a small hole made in the apex, and then immersed in the same fixative. After fixation for 1 hour, the cochleae were rinsed with cacodylate buffer and postfixed with 1% OsO_4 by gentle cochlear perfusion and immersion at 4°C for 1 hour.

The cochleae were dissected under cacodylate buffer to expose the organ of Corti of all turns of the cochlea. In order to determine if erosion of the

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From the Department of Otolaryngology–Head and Neck Surgery, Otology Laboratory, University of California, Davis, California. Supported by grants from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders (R01 NS21079) and National Institute on Aging (R01 AG04959). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Davis.

REPRINTS — Michael D. McGinn, PhD, Otology Laboratory Surge III, University of California, Davis, CA 95616.

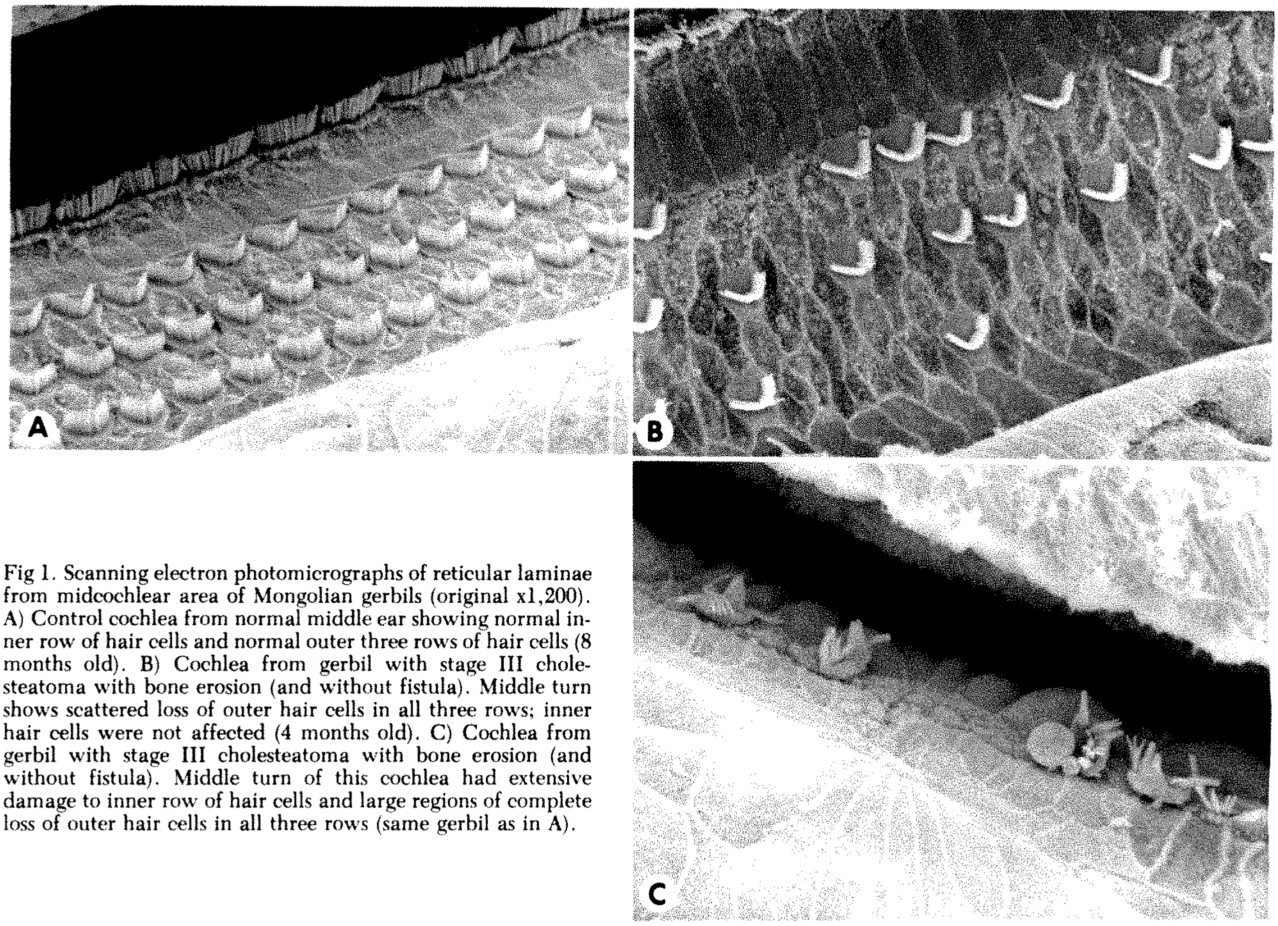

Fig 1. Scanning electron photomicrographs of reticular laminae from midcochlear area of Mongolian gerbils (original x1,200). A) Control cochlea from normal middle ear showing normal inner row of hair cells and normal outer three rows of hair cells (8 months old). B) Cochlea from gerbil with stage III cholesteatoma with bone erosion (and without fistula). Middle turn shows scattered loss of outer hair cells in all three rows; inner hair cells were not affected (4 months old). C) Cochlea from gerbil with stage III cholesteatoma with bone erosion (and without fistula). Middle turn of this cochlea had extensive damage to inner row of hair cells and large regions of complete loss of outer hair cells in all three rows (same gerbil as in A).

cochlear wall had occurred, the cochleae were carefully examined under the operating microscope at high magnification and the surface of the cochlear wall was tested with a small pick. Bone erosion resulted in a roughened surface and thinner, more delicate cochlear walls, particularly in the middle turn. Bone erosion was never seen in cochleae from normal control ears or from ears with cholesteatomas that did not contact the cochlear wall.

Cochleae that had been in contact with stage III cholesteatomas were separated into two categories: those with bone erosion and those without bone erosion. Animals with more extensive cholesteatomas (stage IV or V)[4] or with cochlear fistulae were discarded. This report includes data from 20 gerbils that provided 32 cochleae from either normal control ears or ears with stage III[4] cholesteatomas. Most cochleae were obtained from gerbils with unilateral ligations; cochleae from two control animals and two binaurally ligated animals were also included. Efforts were made to match the numbers and ages of the animals in each of the three groups. Mean percentages were obtained from and statistical analyses were conducted on 25 cochleae: 10 from control ears, 8 from ears with stage III cholesteatoma with bone erosion, and 7 from ears with stage III cholesteatoma without bone erosion.

After dissection, the cochleae were washed in distilled water and immersed in a saturated solution of thiocarbohydrazide for 30 minutes. After an additional wash in distilled water, they were placed into 1% OsO_4 a second time for 2 hours. The thiocarbohydrazide and OsO_4 steps were repeated twice.[5]

The cochleae were critical point dried, mounted on aluminum stubs, and examined on an ISI-DS130 scanning electron microscope. Each region of the organ of Corti was photographed at a magnification of 700x. After photography, the cochleae were further dissected to expose the hair cells of the medial portions of each turn; this process sometimes required multiple dissections.

Hair cell counts were obtained by counting the presence or absence of the hair cell cuticular plate. A hair cell was counted as missing if no cuticular plate was visible. The percentage of inner hair cells and outer hair cells remaining was determined in each of three equal-sized regions along the outer two thirds of the basilar membrane of each of the three cochlear turns. In some cochleae, some regions of the basilar membrane were not available for analysis. To permit the use of repeated measures analyses of variance (ANOVAs),[6] these unavailable regions were assigned the highest percentage of hair cells present in that cochlea. This is a conservative procedure that favors the null hypotheses. Significance levels for the comparisons provided by simple

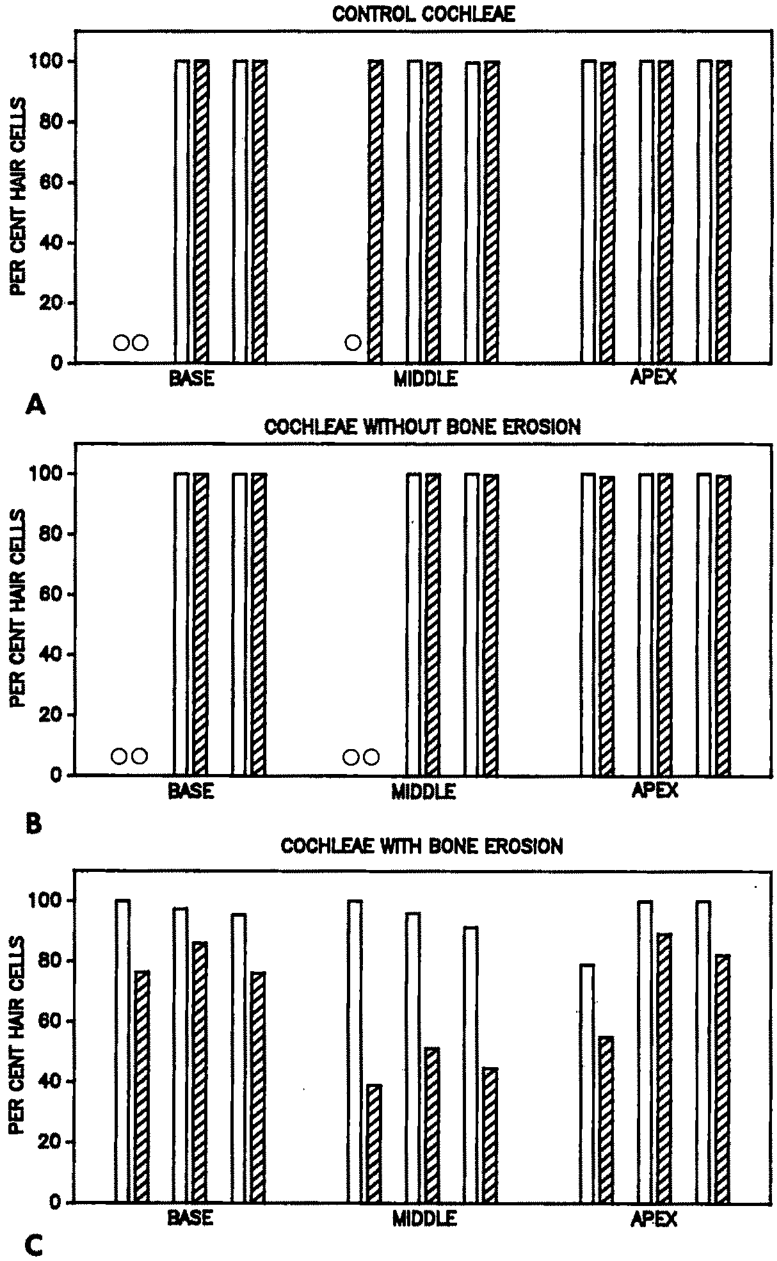

Fig 2. Mean percentages of remaining cochlear hair cells in each of three regions into which each of three cochlear turns was divided. Blank bars — inner hair cells, hatched bars — outer hair cells, blank circles — regions that could not be sampled. A) Cochleae from gerbils with normal middle ears (n = 10). B) Cochleae from gerbils with stage III cholesteatomas without bone erosion (n = 18). C) Cochleae from gerbils with stage III cholesteatomas with bone erosion but without fistulae (n = 7).

main effects analysis were corrected by a sequentially rejective Bonferroni test.[7]

RESULTS

Qualitatively, there was a pronounced loss of hair cells in cochleae from temporal bones with stage III cholesteatomas with bone erosion (and without fistulae; Fig 1). The loss was more severe in outer hair cells than in inner hair cells (Fig 2). The cholesteatomas that did not erode the bone resulted in little loss of hair cells. The cochleae in these latter gerbils did not differ from the cochleae obtained from animals with clear middle ears.

Five cochleae with bone erosion were compared with matched cochleae from clear middle ears. In the control cochleae, all inner hair cells (100%) and most outer hair cells were present (99.0%). In the cochleae with bone erosion, most inner hair cells were present (95.7%), but there was a pronounced loss of outer hair cells (67.6% present).

In a three-way ANOVA, the cochleae from clear middle ears (control), ears with stage III cholesteatomas without erosion of cochlear bone (no erosion), and ears with stage III cholesteatomas with erosion of cochlear bone (erosion) were considered as independent treatments. The percentage of remaining inner hair cells versus the mean percentage of remaining outer hair cells was considered as one set of repeated measures, and the three cochlear turns (base, middle, and apex) were considered the second set of repeated measures.

The ANOVA showed significant overall main effects for treatment ($F_{2,22} = 12.2$; $p = .005$), for the

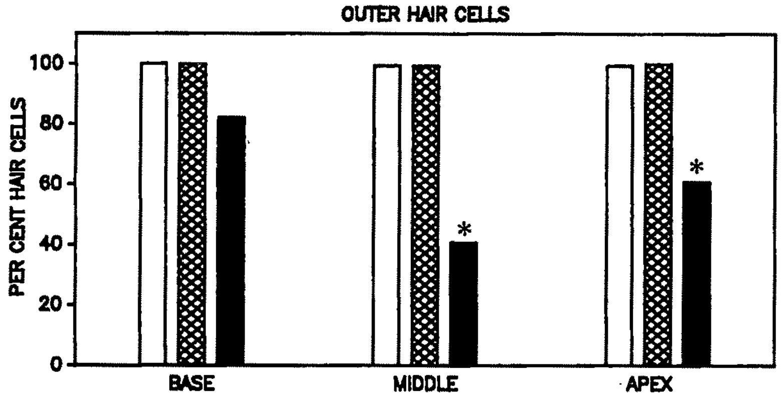

Fig 3. Mean percentage of remaining outer hair cells in each cochlear turn. Open bars — cochleae from gerbils with normal middle ears, crosshatched bars — cochleae from gerbils with stage III cholesteatomas without bone erosion, filled bars — cochleae from gerbils with stage III cholesteatomas with bone erosion but without fistulae, asterisks — mean percentages that differ significantly from those of control cochleae and cochleae without bone erosion at $p < .0001$.

comparison between inner and outer hair cells ($F_{1,22} = 15.0$; $p = .0011$), and for the interaction between all three factors: treatment, inner versus outer hair cells, and cochlear turn ($F_{4,44} = 4.6$; $p = .0036$). Simple main effects analysis showed that the treatment effect was significant only in the middle ($F_{2,70} = 55.4$) and apical turns ($F_{2,70} = 23.5$) of the cochleae with bone erosion ($F_{1,22} = 84.1$). Although in some cochleae with bone erosion inner hair cells were greatly affected (Fig 1C), losses were significant only in outer hair cells ($F_{2,42} = 49.7$; $p < .0001$ for all comparisons; Fig 3).

We examined two gerbils with stage III cholesteatomas that also exhibited severe chronic otitis media. In both animals the bone was thickened in the walls of the cochlea and the bulla. Each of these animals exhibited a 3% loss of outer hair cells: one from the middle turn only and one from the apical turn only. In both animals, the outer hair cells of the basal turn and the inner hair cells of all turns appeared normal. The loss of hair cells in these two animals was not more remarkable than that seen in some controls.

Other than time required for the cholesteatomas to develop, invaginate, and contact the bulla, age did not seem to be an important variable in this study. Four of the stage III cholesteatomas occurred in the youngest group of animals: two in the group with erosion of the cochleae and two in the group without erosion of the cochleae. The greatest mean loss of outer hair cells (69%) occurred in a 3-month-old gerbil, and the second greatest (67%) in a 10-month-old gerbil.

DISCUSSION

Cochlear damage that is sometimes seen in ears with chronic otitis media may be due to several factors. Chronically infected ears may have been treated with multiple courses of potentially ototoxic topical antibiotics.[8] Ototoxic products of the inflammatory process, including lysozymes and arachidonic acid metabolites, may traverse the round window and damage cochlear hair cells.[9] This study raises the possibility that ototoxic substances involved in osteoclastic resorption associated with cholesteatomas, such as prostaglandins, lysozymes, collagenases, and proteinases,[10] may directly affect hair cells subjacent to the region of erosion even without breach of the cochlear wall.

The presence of experimental cholesteatomas alone, without bone erosion, is not associated with cochlear hair cell loss. This observation may be contrasted with the previous findings by Chole and Chiu[2] of apical hair cell loss in early, stage II cholesteatomas. However, the gerbils in this earlier study were older (1 to 2 years of age), and their cholesteatomas were of spontaneous origin, developing later than those experimentally induced. The loss of hair cells in these animals may have been age-related, since the apical turn of older gerbils exhibits age-related damage to the stria vascularis.[11]

When there was bone erosion of the cochlear prominence without fistulae, significant hair cell loss was found in the subjacent middle and apical turns of the cochlea. This probably occurs because the location of the stapedial artery prevents the cochlear wall of the basal turn from contact with the cholesteatoma. If toxic products had been produced by the cholesteatoma itself, or by substances related to intermittent bouts of otitis, we would expect hair cell loss in the region of the cochlea nearest the round window, ie, the basal turn.

We conclude that in gerbils, middle ear cholesteatomas that result in bone erosion are associated with loss of outer hair cells in the middle and apical turns. The loss of cochlear hair cells may be due to diffusion of toxic substances through the bony wall directly into the cochlear turn.

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7TH WORLD CONGRESSES FOR BRONCHOLOGY & BRONCHOESOPHAGOLOGY

The 7th World Congresses for Bronchology & Bronchoesophagology will be held Sept 28-Oct 2, 1992, at the Mayo Clinic and Mayo Medical Center in Rochester, Minnesota. For further information, contact Udaya B. S. Prakash, MD, Secretary-General & Director, 7th WCB & WCBE, East-18, Mayo Clinic, Rochester, MN 55905.

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EFFECT OF VIRUSES AND INTERFERON ON CHICK EMBRYO OTOCYST CULTURES

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Chick embryo otocyst organ cultures were subjected to live vesicular stomatitis virus and rubella virus preparations, to interferon (IFN), and to a combination of both virus and IFN, and compared to control untreated otocysts. We observed morphologic and microscopic changes suggestive of individual cell death and delayed organ differentiation in the virus-treated groups, along with an appreciable decrease in size of the otocyst. Low-dose IFN treatment prior to virus inoculation appeared to partially prevent these effects. The addition of IFN alone did not seem to affect the differentiation process. Time-lapse videophotography further confirmed the above findings. This study suggests that the peripheral component of congenital deafness associated with viral infections is likely to be an effect of the virus itself, and not of the IFN. Interferon provides a partial protective effect against the insult from the virus in vitro and does not seem to be toxic to the developing otocyst.

KEY WORDS — chick embryo, interferon, otocyst, rubella, vesicular stomatitis, virus.

INTRODUCTION

Virus infections in the first trimester of pregnancy have been associated with thousands of cases of congenital hearing loss.[1,2] The exact mechanism by which this occurs is unknown. Interferon (IFN), a substance made in response to virus infection, has recently been shown to specifically inhibit cell differentiation in several systems. Whether the virus infection itself or the immunologic product (such as IFN, made in response to the infection) is responsible for the congenital deafness is not known. It is hence not clear whether IFN is beneficial or detrimental to the young embryo. The present study was undertaken in order to better understand the mechanism of virus-induced congenital hearing loss, to identify agents (such as virus or virally induced products) that can induce morphologic changes in the development of the ear, and to identify agents (such as IFN) that have the potential to protect against such changes. The inner ear anlage (otocyst) of the fowl embryo grown in soft agar was chosen as our model. The otocyst was infected with a virus (vesicular stomatitis virus [VSV] or attenuated rubella virus) with or without addition of IFN to the organ culture. The changes induced by the virus and IFN treatment were studied morphologically by time-lapse photography, and histologically by light microscopy, while we looked for any protective effect of IFN against viral toxicity.

MATERIALS AND METHODS

Culture Technique. Three-day-old fertilized chicken eggs were obtained from Truslow Farm, Chestertown, Md. Microsurgery and explantation were done as previously described.[3-5] The otocysts were dissected under sterile conditions and cultured in semisolid media consisting of 0.75% soft agar in RPMI, 5% fetal bovine serum, 1% penicillin-streptomycin, and 1 μg/mL dexamethazone. Quadriplate otocyst organ cultures were employed in each experiment.

One group of otocysts was subjected to live VSV (a virus known for its cytopathic effects in tissue culture), and another to Meruvax, a vaccine to rubella (a virus clinically associated with congenital hearing loss). A third group was pretreated with IFN for 12 hours and then inoculated with the virus. The fourth group was treated with IFN alone. All these were compared to untreated otocysts. The experiment was stopped after 48 hours in all groups.

Interferon Production. Interferon was produced in allantoic fluid of chick embryos infected at 7 days with WS influenza virus, as described.[6,7] The IFN was pH2-treated for 24 hours, redialyzed against phosphate-buffered saline pH 7.4, and subjected to initial purification with Sephadex chromatography, and pooled active fractions were used for preparation of the laboratory standard. The National Institutes of Health chick IFN standard was obtained for comparison and standardization of assays. The IFN was assayed in chick embryo fibroblasts primary culture by using a VSV plaque reduction. A viral concentration equal to 10^8 particles per milliliter and an IFN concentration equal to one fourth (vol/vol) were found to be optimal. This concentration corresponded to an IFN titer of 40 U/mL stock solution.

RESULTS

The results are based on 30 experiments culturing a total of 180 otocysts. These were equally divided into six groups (VSV, rubella, IFN + VSV, IFN +

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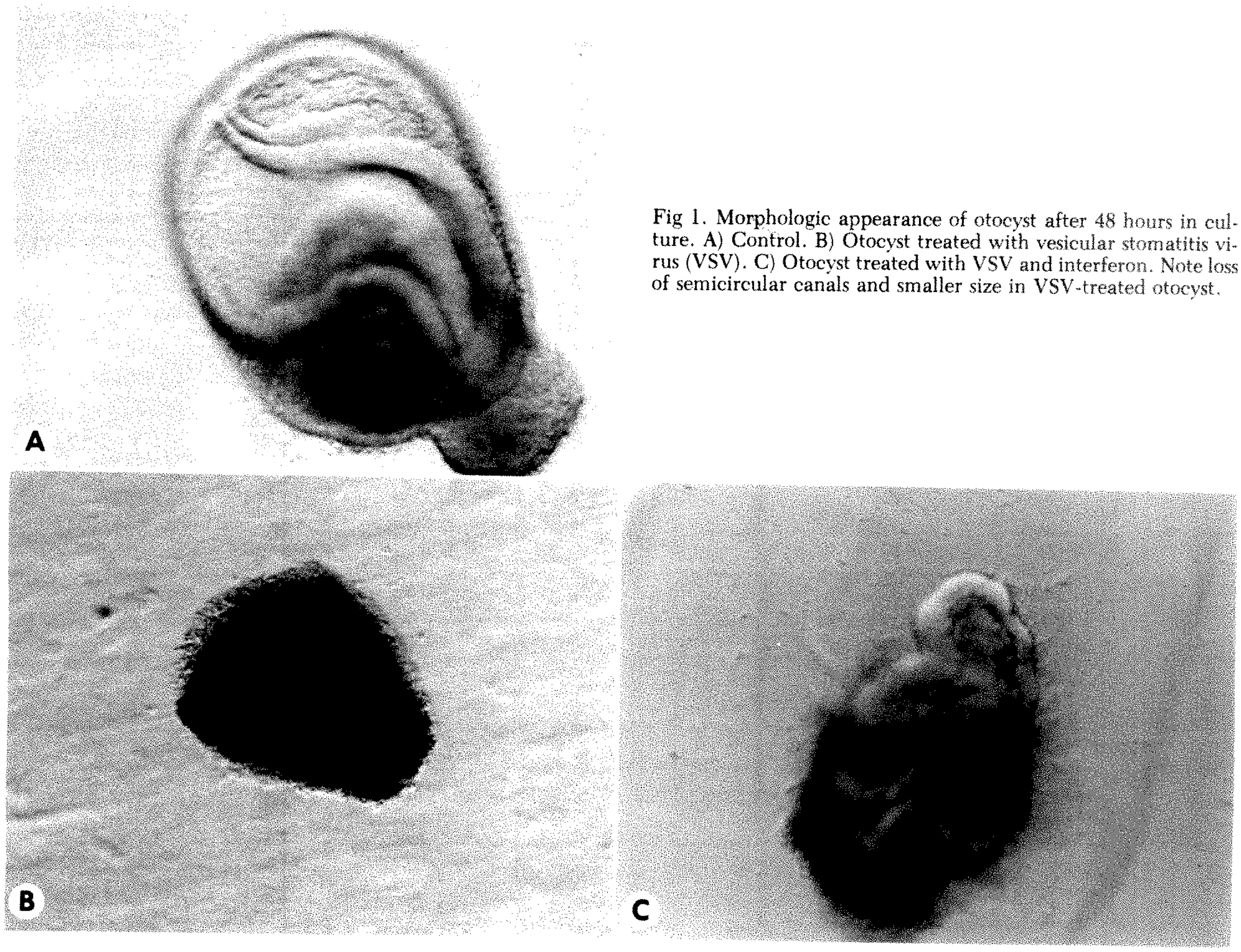

Fig 1. Morphologic appearance of otocyst after 48 hours in culture. A) Control. B) Otocyst treated with vesicular stomatitis virus (VSV). C) Otocyst treated with VSV and interferon. Note loss of semicircular canals and smaller size in VSV-treated otocyst.

rubella, IFN alone, and control). The control otocysts enlarged considerably during their development in vitro, and the isolated spherical 3-day-old otic vesicle developed three semicircular canals within 48 hours. The VSV-treated otocysts initially enlarged in size, but then collapsed approximately 24 hours postinfection. They did not develop semicircular canals, but showed morphologic and histologic changes of vacuolization suggestive of cell death. These changes were not seen in the otocysts pretreated with IFN. Rubella-treated otocysts did enlarge, but to a size significantly smaller than the corresponding control otocysts. The dramatic cytopathic effect seen with VSV was noted to a much lesser extent with rubella. Interferon pretreatment allowed rubella-treated otocysts to develop to full normal size. Treatment of the otocysts with IFN alone did not significantly affect the growth rate, morphologic and histologic appearance, or final size within the time frame and dosage used in the experiment.

Figure 1A shows the gross morphologic appearance of the control, Fig 1B of the VSV-treated otocyst, and Fig 1C of the IFN + VSV–treated otocyst. Figure 2A shows the histology of the control otocyst, Fig 2B of the VSV-treated otocyst, and Fig 2C of the IFN + VSV–treated otocyst.

At 48 hours after inoculation, computer-assisted videophotographs of the otocysts were taken and the surface areas of the otocysts were measured. The mean relative change in size of the otocysts in each group was determined by calculating the ratio of the surface areas at times 48 hours and 0 hours. The results are plotted in Fig 3. The changes between control and VSV groups, VSV and IFN + VSV groups, control and IFN + VSV groups, control and rubella groups, and rubella and IFN + rubella groups are statistically significant to $p < .05$ (control versus VSV, $p = .001$; VSV versus IFN + VSV, $p = .013$; control versus IFN + VSV, $p = .034$; control versus rubella, $p = .035$; rubella versus IFN + rubella, $p = .043$). The changes between the other groups were not statistically significant.

DISCUSSION

Since first developed in 1926, the in vitro culture of the isolated otocyst of the embryonic fowl has been used to study the embryology and morphogenesis of the developing inner ear.[5,8-10]

While the ototoxicity of aminoglycosides antibiotics has been investigated,[11,12] little is known about the mechanisms of viral ototoxicity during the early morphologic maturation. Many viruses (including mumps, rubella, varicella, herpes simplex, small-

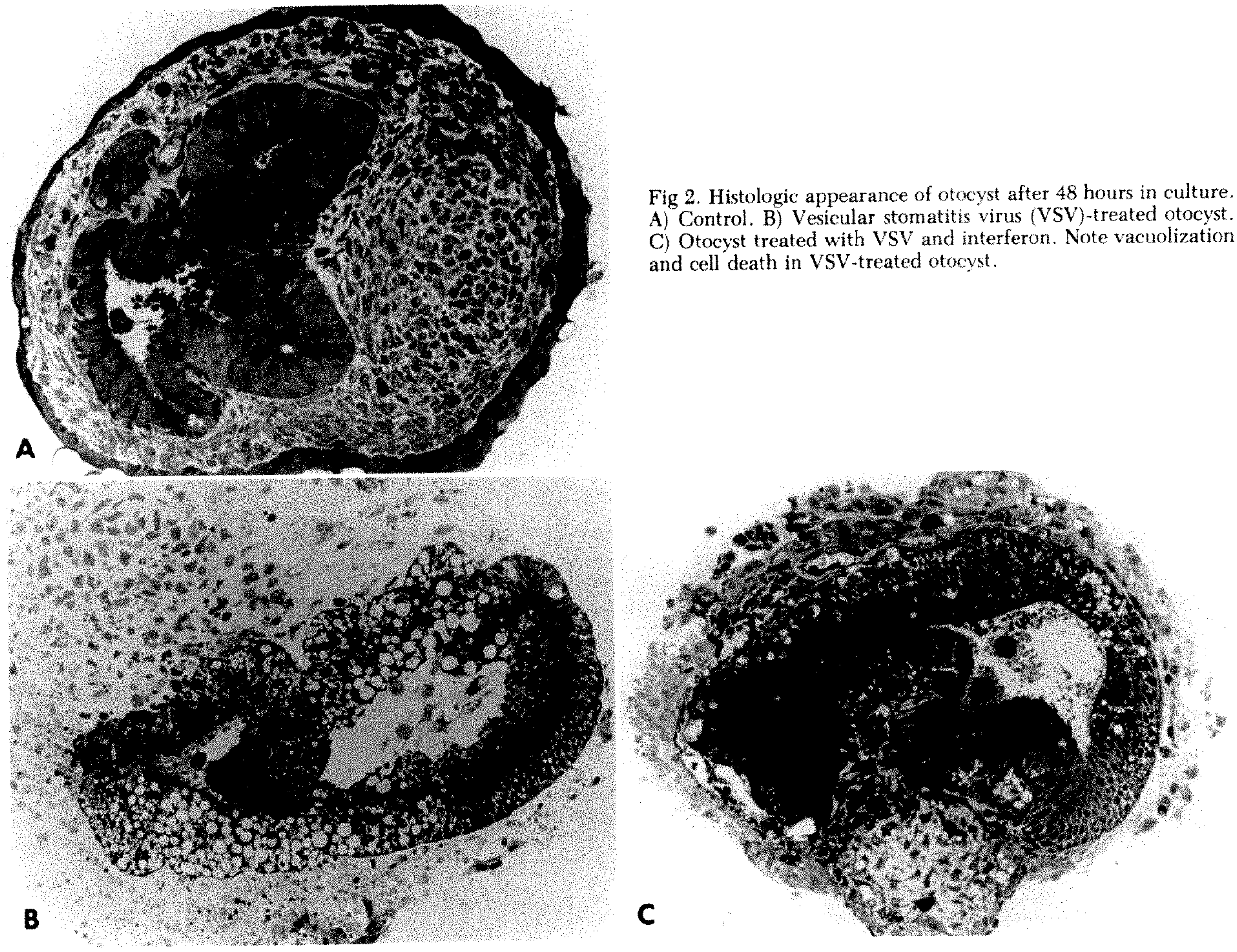

Fig 2. Histologic appearance of otocyst after 48 hours in culture. A) Control. B) Vesicular stomatitis virus (VSV)-treated otocyst. C) Otocyst treated with VSV and interferon. Note vacuolization and cell death in VSV-treated otocyst.

pox, adenoviruses, cytomegaloviruses, and the coxsackie viruses) have been implicated in cases of sensorineural hearing loss.[13,14] Apparently, these viruses are capable of producing an inner ear lesion in the unborn fetus without producing any damage to the inner ear of the mother.[14] Whether the developing embryonic structures are more sensitive to the ototoxic effects of the virus than the mature ear, or whether a secondary immunologic phenomenon associated with the virus infections (such as IFN) is toxic to the fetus without affecting the mother is not known.

Interferons are known to have a direct effect on differentiation. They have been shown to have both enhancing and deleterious effects on cell differentiation in several systems.[15-17] Some of the most striking effects are the inhibition of chick and human myogenesis, that of spontaneous and melanotropin-stimulated melanogenesis, that of insulin-dependent 3T3-L conversion to adipocytes, and that of leukemic cell differentiation.[18,19] On the other hand, IFNs stimulate lymphocyte maturation and differentiation of macrophages and natural killer cells.[20] Little is known about the direct effects on different tissues within the embryo or on an organ system culture such as the otocyst. We have employed partially purified chick IFN, which represents a mixture of alpha and beta IFNs. Our investigations show, as expected, that two viruses have a cytopathic effect on the embryonic tissue development. The VSV-treated otocysts' size decreased, and their architectural differentiation was arrested. Histologically, the infected organs showed many spherical vesicles filled with clear fluid, with "ballooning" of the cy-

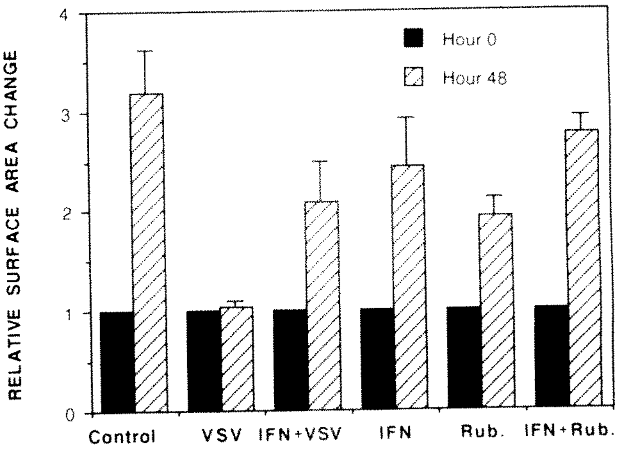

Fig 3. Relative change in size of otocyst after 48 hours in culture. VSV — vesicular stomatitis virus, IFN — interferon, Rub. — rubella virus.

toplasm as seen with virally induced cell death. Unlike naturally occurring infections, the otocyst organ culture employs systemic virus infection with a large inoculum, overwhelming what little immune system may be available to combat infection and resulting in the inevitable death of the embryo. Hence, the variety of cellular responses described with viral infections (such as giant cell formation, inclusion bodies, etc)[21] is not seen here. The rubella-treated otocysts showed a decrease in size and a delay in differentiation, but to a lesser degree than the VSV-treated ones.

Pretreatment with IFN before virus inoculation partially prevents the cytopathic effect of the virus. Differentiation of the semicircular canals seems to continue, although to a lesser extent, and the otocyst remains viable, even beyond the 48-hour end point of the experiment. Interferon alone did not appear to significantly affect differentiation of the otocyst.

We conclude that the congenital hearing loss associated with viral infections is due to the cytopathic effect of the virus itself, and not to the immunologic by-products. Interferon seems to provide protection against the viral insult. In our model, IFN does not appear to be ototoxic to embryonic ears in culture. This may be of clinical interest, in view of the availability of exogenous IFNs as antiviral agents.

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SERIAL CULTURE AND CHARACTERIZATION OF THE CHINCHILLA MIDDLE EAR EPITHELIUM

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We have successfully cultured a fibroblast-free chinchilla middle ear epithelium up to the 10th passage by using conditioned medium or using irradiated 3T3 cells as feeder cells. The cultured epithelial cells assumed a polygonal shape with a cobblestone appearance, indicating tight junction formation. A small number of the cells began to show abnormal morphology, such as indistinct cell boundaries, fibroid appearance, or giant cell formation, as the passage increased, particularly after the 5th passage. These morphologically transformed cells showed positive labeling with an anticytokeratin antibody, which indicated the epithelial origin of these cells. Neither ciliated nor secretory cells were observed in the serially cultured cells. The rate of cell growth slowed after the 7th passage, and after the 11th passage the cells no longer proliferated. Even with the above limitations, these cultured cells can be used for a number of in vitro experiments.

KEY WORDS — cell culture, chinchilla, middle ear epithelium.

INTRODUCTION

With the advent of the cell culture technique, a number of investigators have attempted to culture middle ear epithelium in several different rodent species, such as the rat,[1] guinea pig,[2] gerbil,[3] and chinchilla.[4,5] However, only van Blitterswijk et al[6] reported successful serial culture from the rat middle ear mucosa.

In recent years, serially cultured immortalized cells have been extensively used for identifying and characterizing cell surface receptors; for the study of ion, salt, sugar, and amino acid transport for the characterization of the electrical properties of the cell; for the study of cell-cell interaction; and for biochemical and immunochemical characterization of important cellular proteins.

The main effort of this study was to find a suitable method to successfully maintain a pure epithelial cell line in serial passage of the cell culture. In this study, we have successfully serial-cultured the chinchilla middle ear epithelium and have characterized these cells using light microscopy (LM) and electron microscopy (EM).

MATERIALS AND METHODS

A total of 30 healthy adult chinchillas (350 to 400 g) were used.

Culture Medium. A mixture of three parts high-glucose Dulbecco's modified Eagle's medium and one part Ham's F12 supplemented with 5% fetal bovine serum, 0.4 µg/mL hydrocortisone, 10^{-6} mol/L isoproterenol, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10 ng/mL epithelial growth factor (EGF) was used for serial culture of the epithelial cells. The culture medium for 3T3 cells was the same, but without EGF.

Primary Explants. Primary explants were obtained from the chinchilla middle ear mucosa and plated in 35-mm Petri dishes. The explants were rinsed with phosphate-buffered saline (PBS) containing 100 U/mL penicillin and 100 µg/mL of streptomycin, and were then allowed to adhere to the Petri dishes in a small volume of culture medium without EGF. The medium was changed every day for 4 days, until outgrowth of new cells could be seen, and then was changed twice a week. All cultures were incubated in 10% carbon dioxide at 37°C.

Serial Cultures. Serial cultures were performed by using four different techniques as described below. In all four, the cells were harvested from the explant outgrowth, trypsinized, and plated at 2 x 10^4/mL. The trypsin mixture used was composed of 0.25% trypsin (Gibco), 0.05% ethylenediaminetetraacetic acid (EDTA, JT Baker Chemical), and 0.1% D-glucose (Mallinckrodt). On a 35-mm Petri dish, the growth curve of the cultured cells was es-

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tablished to determine the optimum culture time required for the proposed study using standard medium. The cell growth followed an experimental curve with a lag and a log phase peaking at day 14. The four methods used in this study were 1) irradiated 3T3 feeder cells plated at a density of 4.8 x 10^4/mL along with the epithelial cells,[7] 2) low-calcium medium,[1] 3) serum-free, hormone-supplemented medium,[8] as modified by Harada et al,[5] and 4) the supernatant of the 3T3-conditioned medium added to the subcultured epithelial cells in equal amounts with culture medium.

Feeder Cell Preparation. The mouse fibroblast 3T3 cells were rendered mitotically inactive by irradiating them with 30 Gy of gamma rays from a cesium source. These were seeded at a density of 4.8 x 10^4/mL.

Cytokeratin Staining. Cytokeratin staining was done by the avidin-biotin immunohistochemical staining method. Chinchilla middle ear epithelial cells and 3T3 mouse fibroblasts were grown separately in the chamber slides (Lab-Tek). When confluent, the medium was aspirated and the cells were rinsed with PBS. The slides were then fixed in cold 100% methanol and air dried. They were rinsed again with PBS and then blocked with 10% normal goat serum. The slides were then incubated with the primary antikeratin antibody, followed by the secondary goat or rabbit immunoglobulin G, with washes in between. Finally, they were reacted with the avidin-biotin complex employing the chromogen 3-amino-9-ethylcarbazole (Sigma) as the substrate.

Preparation for Morphological Study. For the purpose of LM, middle ear cells were cultured, examined with a microscope (Zeiss ICM 405), and photographed.

For EM, the cells were grown on the coverslip (Thermanox, Miles Scientific). For scanning electron microscopy (SEM), cells attached to the coverslip were rinsed free of culture medium with PBS (0.01 mol/L pH 7.2) and fixed with 2.5% phosphate-buffered glutaraldehyde for the duration of 1 hour, followed by postfixation in 1% osmic acid in phosphate buffer and 2% tannic acid. Following graded dehydration with ethanol, the specimens were critical point dried with carbon dioxide. They were then sputter-coated with gold and examined by SEM (Hitachi S-570). For transmission electron microscopy (TEM), cells were cultured on the plastic coverslip. The specimens were fixed with 2.5% sodium cacodylate–buffered glutaraldehyde, thoroughly washed with a buffer solution, and postfixed in 0.33% osmic acid in S-collidine buffer for 1 hour. Next, they were dehydrated in graded alcohol and embedded in Epon. Finally, ultrathin sections were cut and double-stained with uranyl acetate and lead citrate, and examined by TEM (Philips 300).

RESULTS

The four different techniques employed in the present study showed various degrees of success. Efforts to culture cells by adding 3T3 feeder cells (method 1) were successful (Fig 1A), but this method was abandoned because of the difficulty in identifying fibroblast contamination. The use of low-calcium medium (method 2) to inhibit fibroblast growth in chinchilla middle ear cells was unsuccessful because it could not adequately support their growth. When we used the serum-free hormone–supplemented medium (method 3), plated cells did not even attach to the culture dish, and when this medium was given to first-passage cells, they detached within 24 hours. Of the four different methods applied, the use of equal amounts of conditioned medium and regular medium (method 4) was the most satisfactory in obtaining a pure culture of fibroblast-free epithelial cells in serial culture. Therefore, the description of passaged cells in this paper was based on the fourth method exclusively.

Primary Culture. As described in Materials and Methods, the explants were initially maintained in culture medium without EGF for 4 days. By the third day in culture, outgrowth of the epithelial cells was observed in most explants (Fig 1B). Any explant showing the presence of fibroblasts in the outgrowth was subjected to precision suction and physically removed. This procedure was critical in minimizing potential fibroblast contamination. From the fifth day on, the explants were given medium with EGF.

First Through Fourth Passages. Again, culture dishes showing any evidence of fibroblasts were flushed with medium to force them to detach. If this procedure failed to dislodge the fibroblasts, the contaminated culture dish was discarded (Fig 1C). Even with this judicious procedure to prevent fibroblast contamination, a small patch of fibroblast growth, although rare, was observed among the monolayer of the epithelial cells. The fibroblasts initially appeared to form a monolayer in confluence, with their cell edges overlapping each other with fine filopodia (Fig 1D). This was in contrast to the epithelial cells, which established cell-to-cell contact by filopodial projections before they established tight junctions leading to the monolayer formation (Fig 1E). The tight junctions appeared to be formed among highly populated cells. Therefore, these areas of high cell density formed patches of cobblestonelike monolayers. The margin of the monolayer appeared to be a growing edge (Fig 1E). The neighboring cells with filopodial contact helped to establish cell junctions, incorporating themselves into the monolayer (Fig 1F). The division of cells in the monolayer appeared to occur exclusively in the center of the patch, and not at the edge.

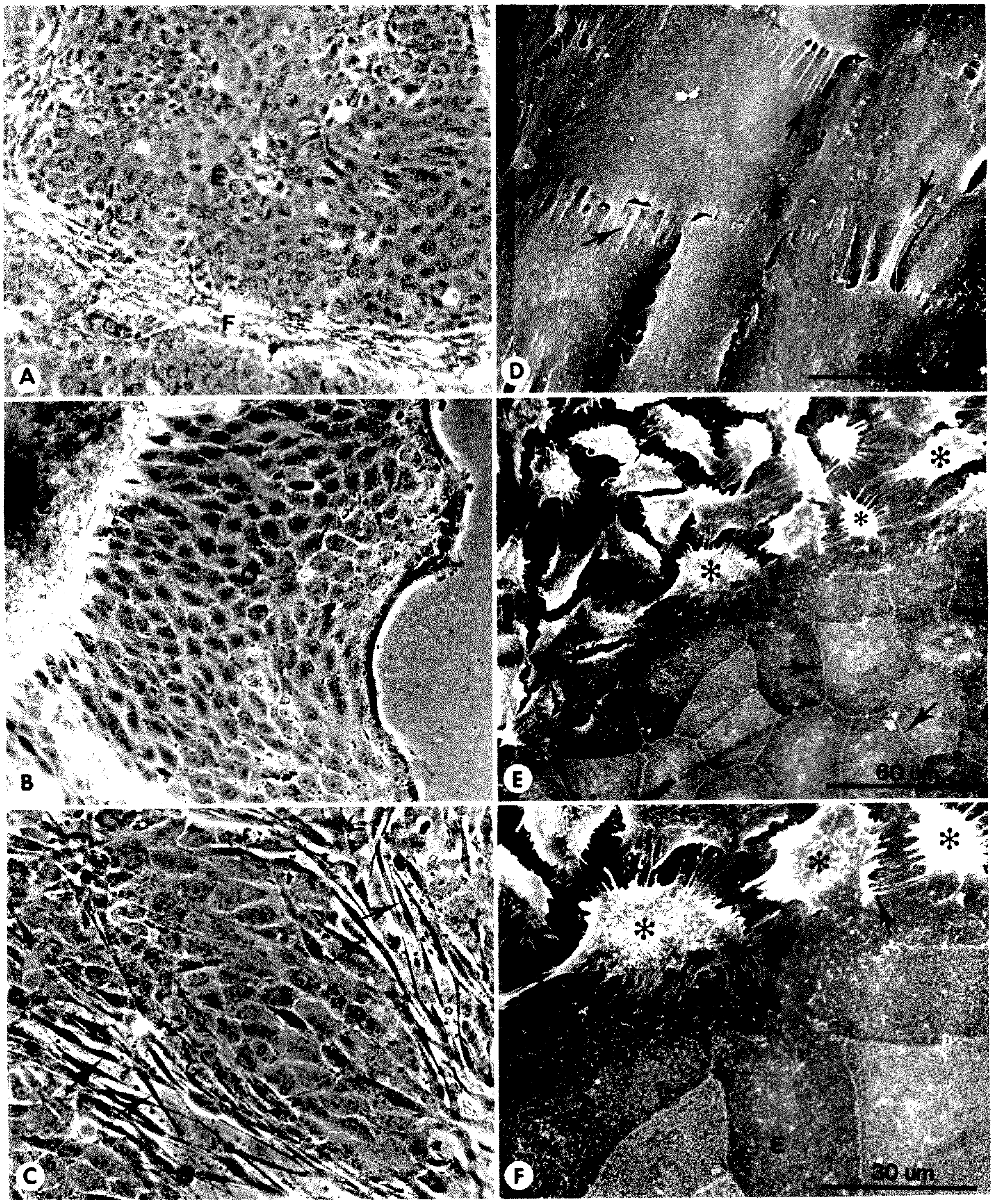

Fig 1. A) Phase-contrast light microscopy photograph showing second-passage epithelial cells (E) after 14 days in culture, surrounded by irradiated 3T3 feeder cells (F; original x125). B) Phase-contrast light microscopy photograph of explant (E) after 3 days in culture, showing epithelial outgrowth (O) that assumes typical cobblestone appearance (original x200). C) Phase-contrast light microscopy photograph of epithelial cells from outgrowth after 7 days in culture, showing spindle-shaped fibroblasts (arrows), which were formed among epithelial monolayer (E; original x200). Such fibroblast-contaminated culture was subjected to suction detachment to physically remove fibroblasts. D) Scanning electron microscopy photograph of second-passage epithelial cells after 14 days in culture, showing patch of fibroblast growth. Edges of these cells overlap each other (arrows). E) Scanning electron microscopy photograph of third-passage epithelial cells after 12 days in culture, forming tight junctions (arrows), which give rise to cobblestone appearance. Newly added cells (asterisks), which possess microspikes (spiny cell projections), are recruited along outer margin of epithelial cell patch. F) Scanning electron microscopy photograph of peripheral area of third-passage epithelial cell patch after 12 days in culture, forming tight junctions. Newly recruited cells (asterisks) form contact with established epithelial cells (E) by using filopodia (arrows).

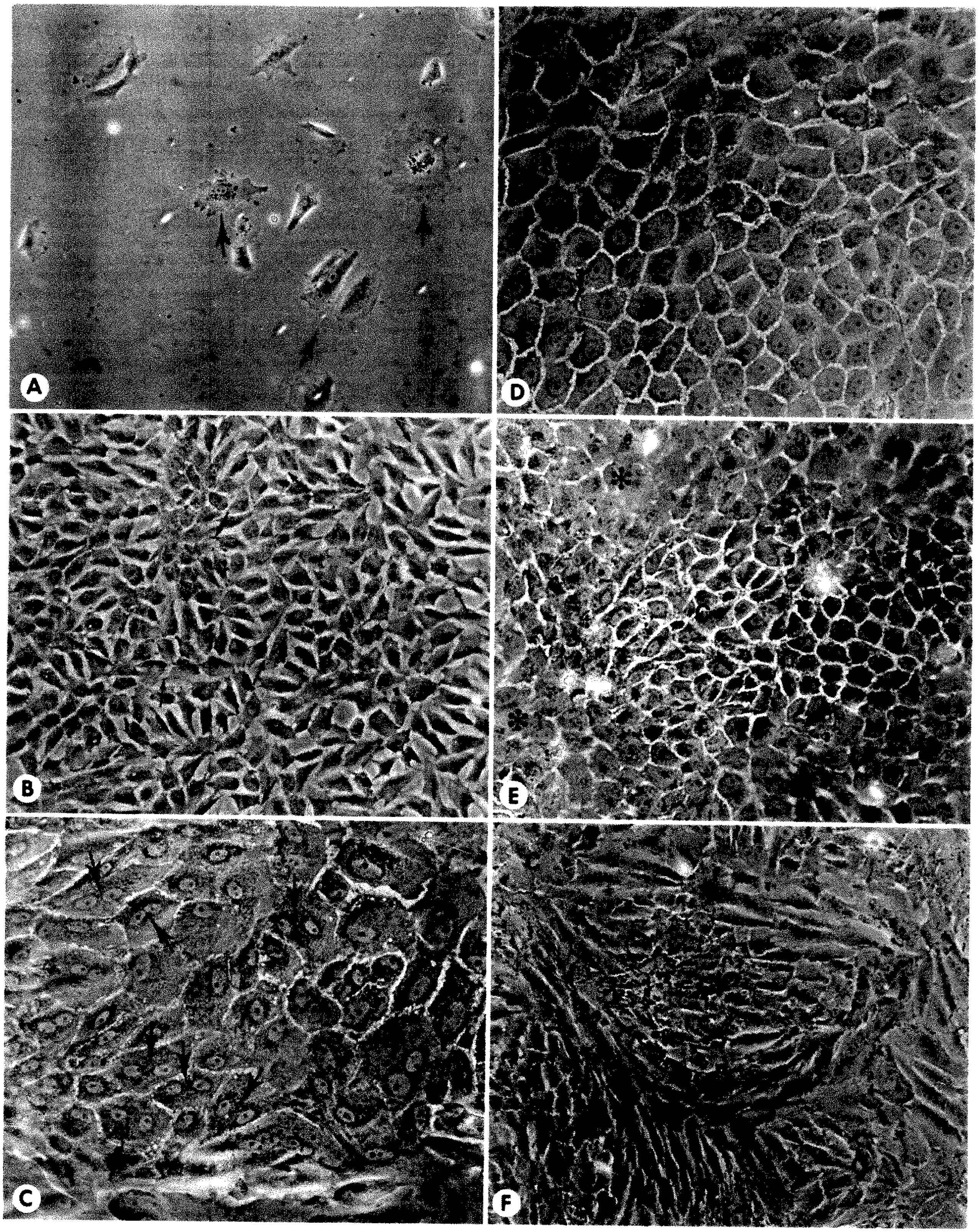

Fig 2. Phase-contrast light microscopy photographs. A) Third-passage cultured epithelial cells showing lamellipodia or ruffled edges (arrows) 5 hours postplating (original x125). B) Third-passage epithelial cells in near-confluence after 6 days in culture, showing cell-cell interaction with filopodia or microspikes (arrows; original x125). C) Fourth-passage epithelial cells after 8 days in culture, showing double nuclei (arrows; original x200). These cells are highly granulated and are interpreted to be dividing cells. D) Fourth-passage epithelial cells after 10 days in culture, showing cobblestonelike formation, which is result of tight junction formation of epithelial cells in confluence (original x200). E) Fourth-passage epithelial cells after 11 days in culture, showing clusters of cobblestonelike cells (C) surrounded by cells with poorly defined cell junctions (asterisks; original x200). F) Fifth-passage epithelial cells after 9 days in culture, showing cells with cobblestone appearance (C) and cells with morphologic changes and indistinct cell junctions (asterisks; original x125). Some of these cells appear fibroid.

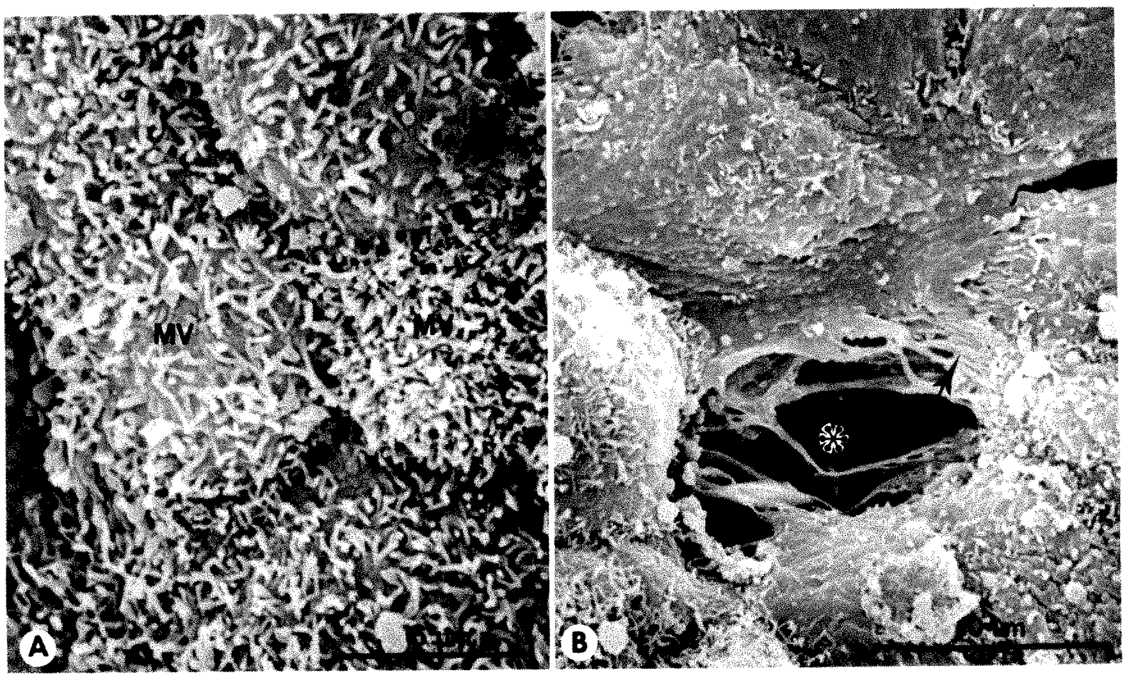

Fig 3. Scanning electron microscopy photographs. A) Fifth-passage epithelial cells with numerous microvilli (MV) after 12 days in culture, showing intimate contact with neighboring cells. B) Fifth-passage epithelial cells after 12 days in culture, showing hole (asterisk) between cells due to incomplete tight junction formation. These cells establish contact with interdigitating filopodia (arrow).

Serial cultures were obtained by trypsinizing the cells with trypsin-EDTA. When trypsinized, the cells became spherical but flattened out in about 8 hours, and growth was demonstrated by the ruffled edges of these cells (Fig 2A). By the sixth day, the number of cells grew dramatically to fill the Petri dish (Fig 2B). By the seventh day postplating (fourth passage), most of the cells became large and had characteristic double nuclei, indicative of mitotic division (Fig 2C). These cells contained various degrees of granulated structures, which were interpreted to be mostly mitochondria. At about 10 days postplating (fourth passage), these large granulated cells formed a confluent layer of cobblestonelike cells (Fig 2D). By the 11th day postplating (fourth passage), clusters of cobblestonelike cells were surrounded by larger cells with less distinct cell junctions (or margins; Fig 2E).

Ciliated cells observed in the outgrowth from the explant were not observed even in the first passage. Likewise, the secretory cells were not observed in serially cultured cells, even though these cells often showed numerous electron-dense granules in an EM examination. These were interpreted to be lysosomal granules.

Fifth Through Tenth Passages. The use of conditioned medium (method 4), particularly at these passages, was of definite advantage over the technique of adding a feeder layer to the culture dish (method 1). With the absence of the feeder layer, it was much easier to spot fibroblasts before they overtook the culture. No significant difference was observed between the growth of the epithelial cells with the feeder layer and that with the conditioned medium.

At passage 5, the cells that had maintained a typical polygonal shape up to the fourth passage underwent a morphologic change and became large and elongated, resembling fibroblasts (Fig 2F). These cells (from the sixth passage) were attached to each other by indistinct cell boundaries (Fig 3A) and interdigitating filopodial processes (Fig 3B).

Even with this problem, serial cultures of the chinchilla middle ear epithelial cells were successfully maintained until the 10th passage (Fig 4). Further attempts to passage these cells were unsuccessful. Even though the cells attached to the dish and established cell-cell contact via filopodial projections, the rate of cell proliferation was greatly reduced (Fig 4A). Therefore, with increasing passage, the cultured cells changed their morphology and lost their ability to form a cobblestonelike monolayer. These cells appeared fibroid and tended to aggregate without clear cell boundaries (Fig 4B), making it difficult to differentiate them from the fibroblasts. To rule out the possibility of fibroblast contamination, 5th-passage cells were stained with cytokeratin. These fibroblastlike cells expressed both cytokeratin and keratin, indicating that these cells were epithelial (Fig 5).

DISCUSSION

Of the four methods used, the conditioned medium, prepared from supernatant of the irradiated 3T3 cells in culture (method 4), provided the best results in obtaining a pure culture of fibroblast-free

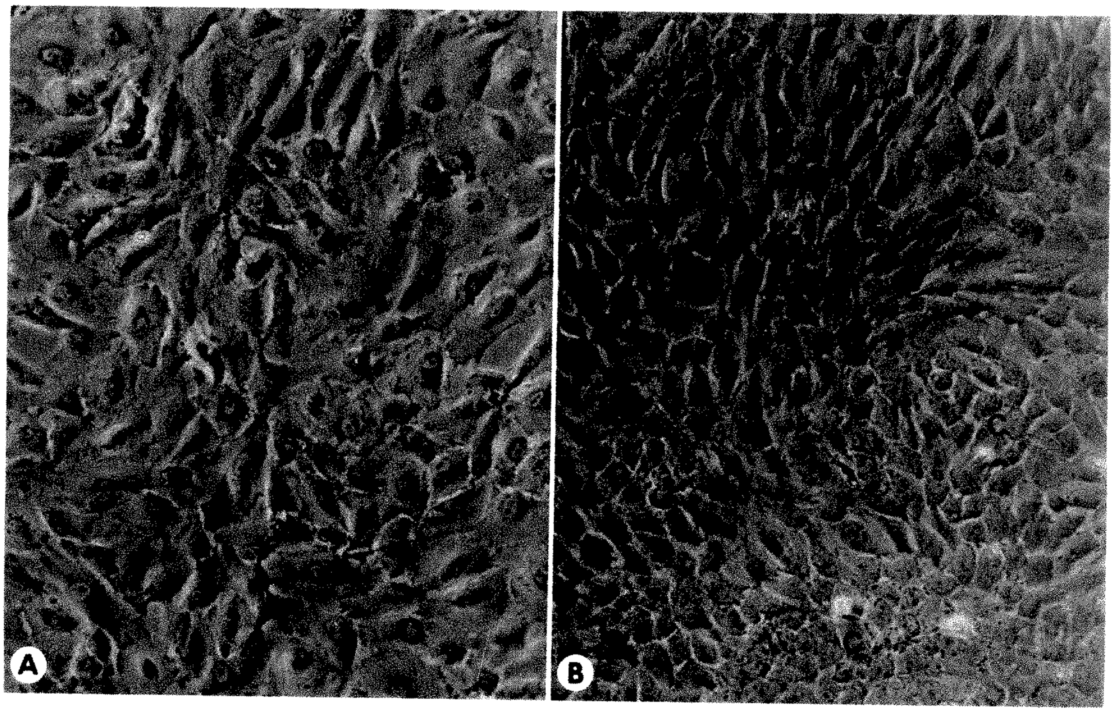

Fig 4. Phase-contrast light microscopy photographs (original x125). A) Tenth-passage epithelial cells after 16 days in culture, showing near-confluence. This delayed confluence is interpreted to be due to slowed cell division. B) Seventh-passage epithelial cells after 10 days in culture, showing large number of morphologically changed cells (asterisks) coexisting with cells forming cobblestone appearance (C). These morphologically altered cells appear fibroid and tend to aggregate without clear cell boundaries.

epithelial cells from the primary explant of the chinchilla middle ear. Because the 3T3 cells are fibroblasts, the use of these cells as a feeder (method 1) made it difficult to identify early chinchilla fibroblast contamination. The use of the low-calcium medium (method 2), successfully used in culturing rat middle ear epithelium,[1] did not support chinchilla middle ear epithelium. The serum-free hormone–supplemented medium (method 3), used for the primary culture of chinchilla middle ear epithelium by Harada et al,[5] did not allow attachment of the plated cells to the culture dish.

The reason for the efficacy of the supernatant of irradiated 3T3 cells in culture for promoting epithelial cell growth is not completely understood. However, it is generally believed that the conditioning of the substrate by the irradiated 3T3 cell products (such as EGF) enhances epithelial cell growth.[9] Further study is needed to clarify the exact nature of the factors in the supernatant of the feeder cells that enhance epithelial cell growth.

Ciliated cells were not observed in the serial culture. The fact that even in primary culture the number of ciliated cells declines with increasing duration in culture can be interpreted to mean that the newly formed cells do not become ciliated. This also implies that basal cells (or nonciliated cells) are the stem cells that provide for the growing cell population and that the migrated epithelial cells are terminally differentiated and may no longer divide.

Well-characterized secretory cells were not observed in the present study, as in our previous study of middle ear explants and primary cultures.[4] Harada et al[5] were able to demonstrate secretory activity through the first passage, but not in the second passage. This limited success in maintaining secretory activity of cultured cells may be due to the addition of retinoic acid in their culture medium. This speculation is largely based on our observation that the secretory cells are successfully maintained in organ culture for 3 weeks when a vitamin A–supplemented medium is used.[10]

The "dome" formation frequently observed in primary culture of the chinchilla middle ear epithelium[4] was rarely observed in serially cultured cells. The dome is an elevated monolayer of cultured epithelial cells formed by the collection of fluid under the epithelium, which indicates that these cells are engaged in active ion and fluid transportation. The paucity of the dome formation in serial cultures implies that these serially cultured cells are no longer able to transport ions and water or to maintain their barrier function. Although the exact mechanisms are not clear, three possibilities may be considered: first, that the tight junction is leaky or fails to be formed by these cells; second, that the lack of polarity of these epithelial cells is due to their inability to form tight junctions, which may lead to failure to segregate the basolateral wall from the cell surface; and third, that the change of cell surface (such as surface glycoprotein) or cell membrane structures

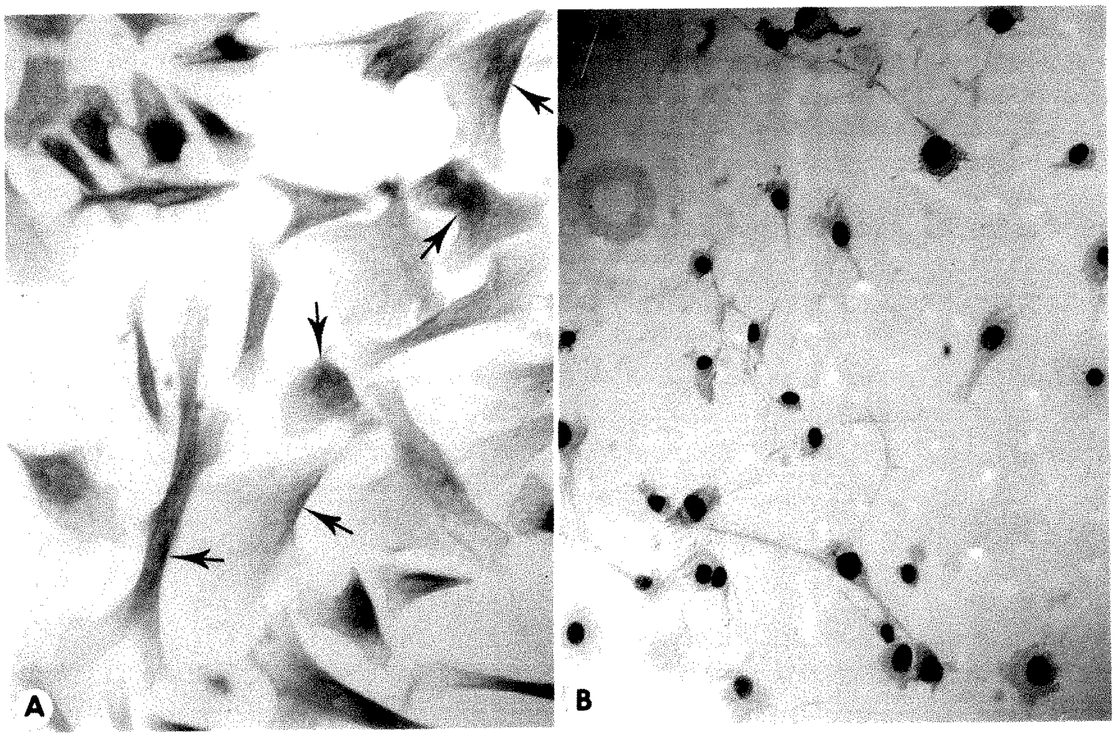

Fig 5. Interference-contrast light microscopy photographs. A) Morphologically changed fifth-passage cells, showing positive immunostaining (arrows) with polyclonal antibody to pancytokeratin (original x200). All cells are stained positive. B) Mouse 3T3 fibroblasts, showing negative immunoreaction with anticytokeratin antibody (original x400). None of cells are stained.

occurs in the basolateral wall that supports the fluid transport function. We have no definitive proof that these cell transformations are caused by surface modification. Further studies are needed to clarify these questions.

A potential drawback of the present serial culture technique is the spontaneous change of cell morphology (transformation) in a small number of cells. The term transformation here is used to denote phenotypic modification of cells without introduction of other genetic materials. It is well known that cultured cells may be transformed by oncogene, virus, or spontaneous uptake of genetic materials.[11] Because the number of transformed cells grew with the increase of passage, one can speculate that these morphologic changes may be due to the advancing age of the cells. The appearance of these transformed cells resembles that of the fibroblast. However, cytokeratin immunostains confirm that these transformed cells are of epithelial origin. The fibroid cell shape in these cells is somewhat similar to that observed in primary cultures of rabbit kidney tubule cells maintained in a culture medium that consists of Dulbecco's modified Eagle's medium and Ham's F12 (50:50 mix) supplemented with 10% fetal calf serum.[12] However, the same cell reached confluence with a cobblestone appearance in serum-free medium.[12]

Failure of the serially cultured epithelial cells to form a cobblestone formation resulted in the presence of abnormal-looking (transformed) epithelial cells, which may be due to the reduction of contact inhibition by these cells. Although the present study could not unequivocally confirm that contact inhibition is truly reduced, the contact inhibition by these cells, if it indeed occurred, may be the result of cell surface modification (eg, changes in the binding of plant lectins and surface glycoproteins, or loss of fibronectin from the cell surface), which is known to occur in transformed cells.[11]

The present technique has a limitation in its inability to maintain ciliated cells and secretory activity in the cultured cells. However, excellent cell morphology is maintained until the fifth passage, after which the cells begin to transform, thus losing their typical polygonal epithelial cell shape. In conclusion, the present techniques using the conditioned medium allowed us to successfully maintain serial culture of the chinchilla middle ear epithelial cells up to 10 passages.

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AMERICAN RHINOLOGIC SOCIETY RESIDENT'S CASE OF THE YEAR AWARD

The American Rhinologic Society announces the Resident's Case of the Year Award. This contest is open to any resident presenting an interesting case related to rhinology. The deadline is January 1, 1992. Case reports should be sent to: Dr George Facer, Secretary, American Rhinologic Society, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. For more information, call (507) 284-3410.

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AESTHETIC SURGERY OF THE FACE

A symposium on Aesthetic Surgery of the Face will be held March 26-28, 1992, in San Francisco, California. This meeting is sponsored by the Division of Plastic Surgery, University of California, San Francisco, and Davies Medical Center. For further information, contact Ashley Carpenter, Extended Programs in Medical Education, Room LS-105, USCF, San Francisco, CA 94143; (415) 476-4251.

Ann Otol Rhinol Laryngol 100:1991

IMAGING CASE STUDY OF THE MONTH

PERINEURAL EXTENSION OF SQUAMOUS CELL CARCINOMA

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INTRODUCTION

Perineural extension can significantly alter the prognosis and treatment of squamous cell carcinoma of the lower lip.[1] Historically, identification of this type of tumor invasion has been difficult owing to the limitations of clinical examination,[2] plain ra-

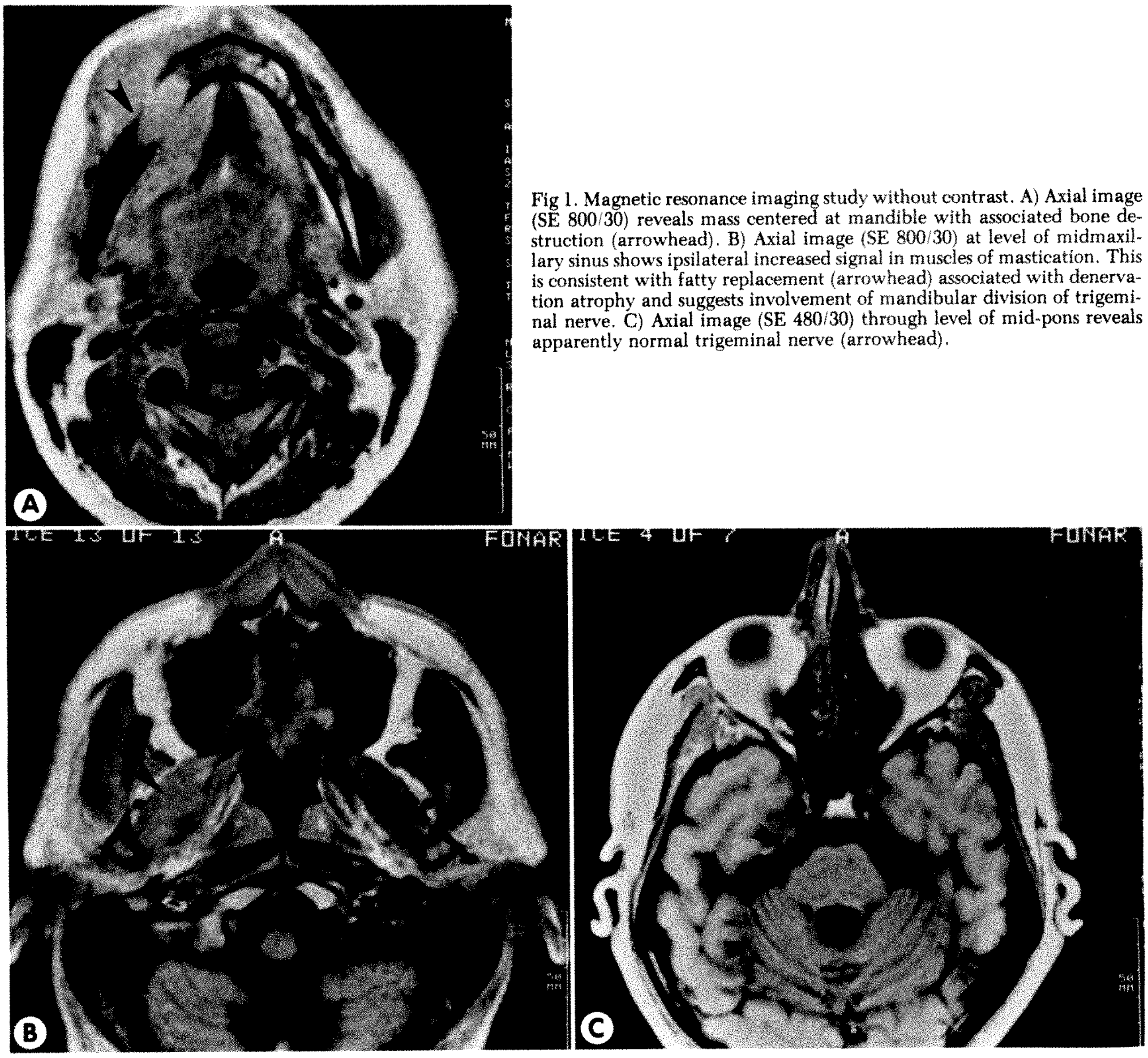

Fig 1. Magnetic resonance imaging study without contrast. A) Axial image (SE 800/30) reveals mass centered at mandible with associated bone destruction (arrowhead). B) Axial image (SE 800/30) at level of midmaxillary sinus shows ipsilateral increased signal in muscles of mastication. This is consistent with fatty replacement (arrowhead) associated with denervation atrophy and suggests involvement of mandibular division of trigeminal nerve. C) Axial image (SE 480/30) through level of mid-pons reveals apparently normal trigeminal nerve (arrowhead).

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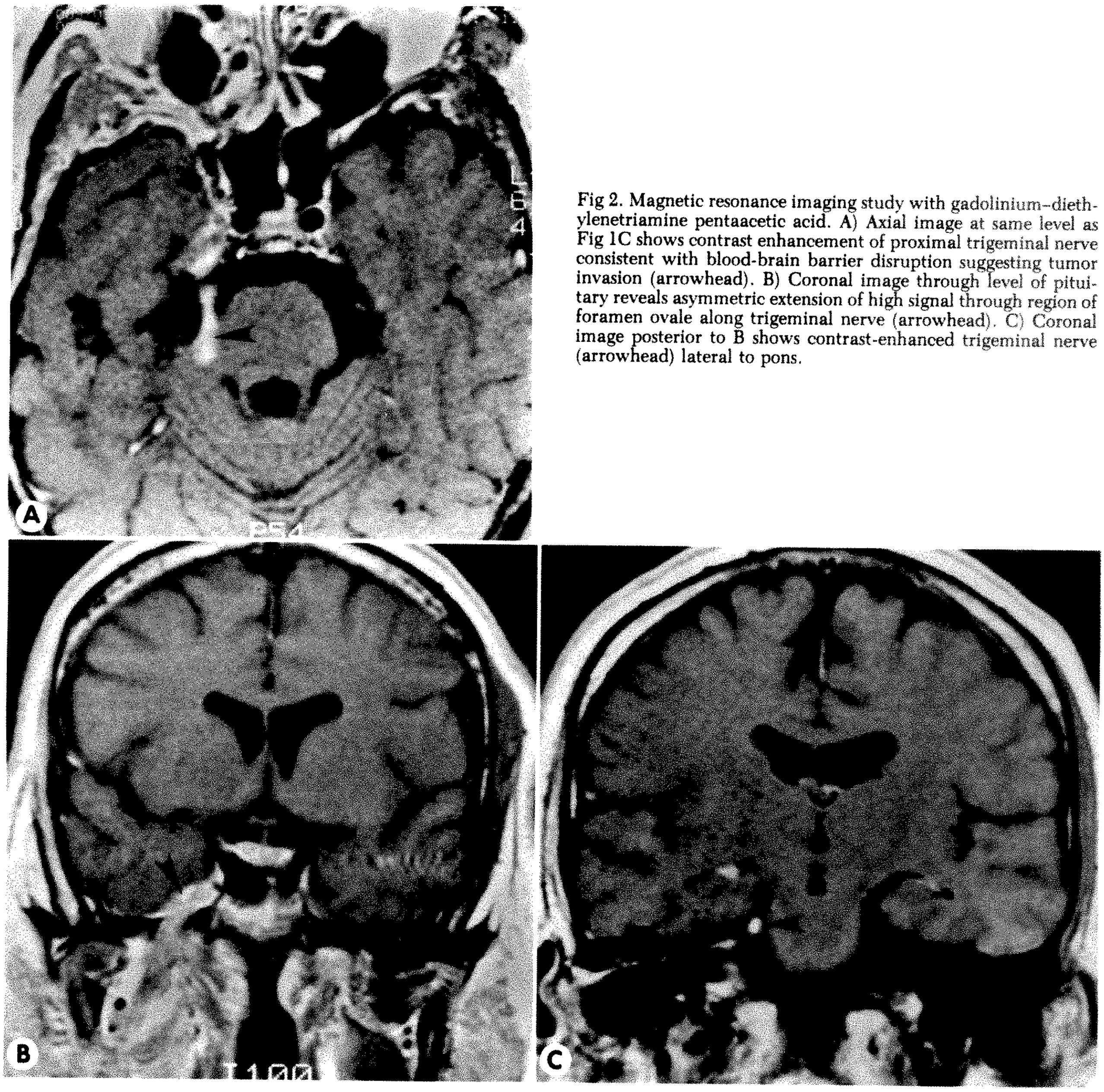

Fig 2. Magnetic resonance imaging study with gadolinium–diethylenetriamine pentaacetic acid. A) Axial image at same level as Fig 1C shows contrast enhancement of proximal trigeminal nerve consistent with blood-brain barrier disruption suggesting tumor invasion (arrowhead). B) Coronal image through level of pituitary reveals asymmetric extension of high signal through region of foramen ovale along trigeminal nerve (arrowhead). C) Coronal image posterior to B shows contrast-enhanced trigeminal nerve (arrowhead) lateral to pons.

diographs,[3] and computed tomographic scanning.[4] Gadolinium–diethylenetriamine pentaacetic acid (Gd-DTPA)–enhanced magnetic resonance imaging (MRI) demonstrates abnormally intense signal along a nerve involved by perineural invasion.[2,4,5] The following case illustrates this enhanced signal along the trigeminal nerve due to perineural extension of squamous cell carcinoma of the lower lip.

CASE REPORT

A 41-year-old man presented with recurrent squamous cell carcinoma of the right lower lip. Two years earlier he had undergone wedge resection of a small squamous cell carcinoma of the right vermilion border. One year later he noted decreased sensation in his right lower jaw. Trismus was noted, but no mass or lesion was identified. Subsequently, the patient developed a nodule of the right buccal cavity that grew to 5 or 6 cm and an ulcerating lesion of the right lower lip. Biopsy revealed recurrent squamous cell carcinoma of the lower lip. A right modified radical neck dissection was performed with composite resection of the hemimandible, the right lower lip, and the floor of the mouth. Reconstruction of the floor of the mouth with the right pectoralis major muscle and a commissure reconstruction with an Abbe-Estlander flap were performed. Initial pathology revealed tumor-free margins and no malignant nodes. After MRI correlation, subsequent reexamination of the mandible in cross section revealed squamous cell carcinoma around and invading the inferior alveolar nerve.

MAGNETIC RESONANCE IMAGING

The MRI scans reveal perineural extension of the squamous cell carcinoma along the right trigeminal nerve. A large mass is seen centered at the right mandible with discontinuity of the dark rim of the mandibular cortex (Fig 1A). With invasion of the inferior alveolar nerve, retrograde involvement of the mandibular division of the trigeminal nerve can result in denervation atrophy of the muscles of mastication. This causes fatty replacement manifesting as ipsilateral asymmetric high signal in the muscles of mastication on the T1-weighted image (Fig 1B).

Direct visualization of perineural extension can be difficult (Fig 1C). However, enhancement after intravenous Gd-DTPA administration makes involvement readily apparent (Fig 2A). The course of the enhanced trigeminal nerve can be followed through the foramen ovale (Fig 2B) and into the pons (Fig 2A,C).

DISCUSSION

Squamous cell carcinoma is the most common malignancy of the lower lip.[6] Byers et al[1] showed a 2% incidence of perineural invasion in 1,308 cases of squamous cell carcinoma of the lower lip. Growth of the tumor along perineural lymphatics can progress to extension through the mental foramen and invasion of the inferior alveolar nerve. More proximal involvement via the mandibular division of the trigeminal nerve can traverse the base of the skull through the foramen ovale.[7] This spread permits intracranial involvement of the gasserian ganglion in Meckel's cave, the preganglionic trigeminal trunk in the prepontine cistern, and the lateral pons.

Perineural invasion can significantly alter the treatment and prognosis of squamous cell carcinoma of the lower lip.[1] Treatment of small lesions without nodal metastases usually involves local surgical excision.[7] Perineural invasion can necessitate wider excision and may require hemimandibulectomy and radical neck dissection. In advanced perineural extension, even aggressive surgical resection may fail to yield tumor-free margins and may require postoperative radiotherapy.[3,7] The poor prognosis is especially bad in the presence of associated cervical nodal metastases and a tumor size greater than 3 cm.[1,8]

Magnetic resonance imaging is an effective modality for imaging the head and neck region.[4,5,9,10] Superior soft tissue contrast, multiplanar capability, and absence of artifact from dental amalgam, skull base, and mandible allow confident evaluation of head and neck disease.

Gadolinium DTPA is a paramagnetic MRI contrast agent that shortens T1 (and to some extent T2) relaxation times of tissue in which it accumulates. It particularly improves the MRI conspicuity of tumors of the nasal cavity and paranasal sinuses and tumors that have intracranial or perineural extension.[2,4,5]

Perineural tumor extension is a difficult problem in the management of head and neck malignancy. Patients with squamous cell carcinomas of the lower lip with histologically clear postresection margins can later develop tumor extension above the base of the skull.[3] Clinical signs such as the numbness and trismus exhibited by the patient presented suggest neural involvement. However, clinical signs and symptoms are notoriously inaccurate for lesion localization along the trigeminal nerve.[2]

This case report demonstrates the ability to identify perineural involvement as increased signal on Gd-DTPA-enhanced T1-weighted magnetic resonance images. The potential for discovering early perineural extension in clinically unsuspected or confusing cases suggests the valuable contribution Gd-DTPA-enhanced MRI can make in the management of squamous cell carcinomas of the head and neck.

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PATHOLOGY CONSULTATION

STENSEN'S DUCT CARCINOMAS

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Stensen's duct is rarely a primary site for a salivary neoplasm (11 examples in the English-language literature from 1927 to 1984). Bereft of myoepithelium, the neoplasms are histologically malignant and have been of mucoepidermoid, squamous, or undifferentiated types. Surgical management is predicated on the location and stage of the carcinoma.

The human parotid duct, known also as Stensen's duct, is 6 to 8 cm long in the adult.[1] It begins at the anterior part of the parotid gland, crosses the masseter muscle, and at the muscle's anterior border turns medially, nearly at a right angle, and passes through the fibroadipose tissue of the cheek and pierces the buccinator muscle (Fig 1). The duct runs obliquely forward for a short distance (1 cm) between the buccinator muscle and the mucosa of the oral cavity and opens onto the oral surface of the cheek by a small orifice opposite the second upper molar tooth.[1]

The duct's histoarchitecture is much like that of any other excretory passage that has a peristaltic activity, ie, mucosa, muscular layer, and adventitia. The mucosa is composed of a thin (two to four cell layers) epithelial layer, basement membrane, and a thin subepithelial fibrous connective tissue layer. The luminal epithelial cells are tall columnar cells with various numbers of cilia. Cuboidal cells with large nuclei and a smooth basal surface form the basal cell layer. Goblet cells are occasionally found, but are markedly increased when there is chronic inflammation present.[1]

Neoplasms of Stensen's duct are rare, are almost always histologically malignant, and because of the course of the duct from the parotid gland to the buccal mucosa, can be confused with salivary neoplasms of the oral and buccal soft tissue salivary glands, the accessory parotid gland,[2] or the parotid gland itself.

The most comprehensive study of carcinomas of Stensen's duct is that by Frechette et al,[3] who accepted only 11 examples reported in the English-language literature between 1927 and 1984. Befitting an origin from the excretory part of the salivary duct unit, none of the neoplasms have a myoepithelial component — hence the absence of acinic cell and adenoid cystic carcinomas and pleomorphic adenomas.[4] Frechette et al[3] record six squamous cell carcinomas, four mucoepidermoid carcinomas, and one undifferentiated carcinoma as primary Stensen's duct malignancies (Fig 2).

A carcinoma of Stensen's duct has a variable clinical presentation: as a swelling of the cheek, as a

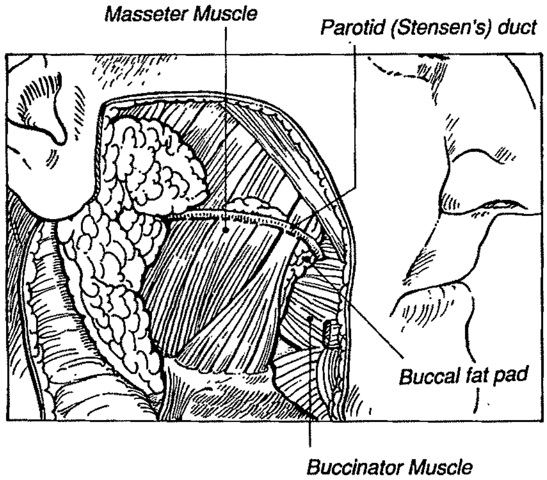

Fig 1. Diagrammatic representation of parotid (Stensen's) duct's anatomic relationships.

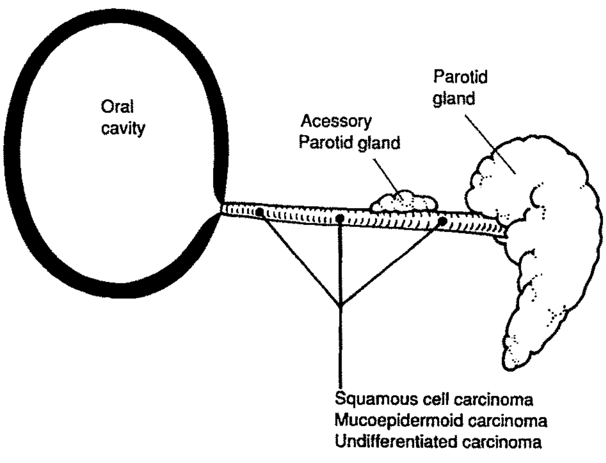

Fig 2. Schematic depiction of Stensen's duct, oral cavity, accessory parotid gland, and parotid gland. Three reported types of Stensen's duct carcinoma must be distinguished from similar carcinomas arising from adjacent epithelial tissues.

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mass within the buccal sulcus, or like a recurrent parotitis with duct obstruction. Depending on delay in diagnosis and the growth pattern of the carcinomas, they may be localized to the duct or infiltrative into soft tissues (buccal, muscle, parotid gland). On the basis of these properties Frechette et al[3] categorize treatment according to stage as indicated by clinical and operative findings. Their group I represents T1 stage I carcinomas, confined to the duct. Group II includes T2 to T3 stage II to III carcinomas that involve the cheek by local invasion. The T4 stage IV neoplasms with extensive local invasion and/or distant metastases are in group III.

Group II and III carcinomas are treated in accord with the accepted principles for parotid carcinomas.[3] Localized carcinomas are excised with the entire duct and at least the lateral lobe of the parotid gland.[3]

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LETTERS TO THE EDITOR

To the Editor:

I would like to comment on the article by Brook and Foote entitled "Microbiology of 'Normal' Tonsils" (*Ann Otol Rhinol Laryngol* 1990;99:980-3). First, I have a considerable difficulty with the definition of "normal" tonsils in this study. I have previously reported on the subject[1,2] and would suggest that "normal" tonsils are tonsils that are small (have not undergone hyperplasia) and have not experienced recurrent or chronic infection.

In this study by Brook and Foote, tonsils were removed that were causing velopharyngeal insufficiency, and although they had not been recurrently infected, I think by definition these tonsils must have been large enough to extend upward behind the soft palate. I am also concerned that they only had four specimens in the "normal" group. The exact size of the tonsils was not reported, neither by weight nor by measurements, and therefore I would like to know how these were determined to be of "normal" size. A study by Sasaki and Koss[3] reported on the bacteriology of normal tonsils that were removed as part of "access surgery" in adults, and the size could have been compared to that of the tonsils in this study.

We published two articles in 1988 in regard to the bacteriology of core biopsy samples of "normal" tonsils.[1,2] We did a core biopsy of "normal" tonsils of children who were undergoing unrelated surgical procedures. The definition of normal tonsils was tonsils that were small, ie, contained by the tonsillar fossae. A core biopsy was done, and our findings were markedly different from those of Brook and Foote. We found that relatively few aerobic bacteria (we did not culture for anaerobes) were present, and those that were were in very small concentrations. (We had 10 control specimens that were in both studies and an additional 2 for our second study.) We found that the tonsil core biopsy of 10 control patients yielded only eight isolates for a mean of 0.8 isolates per patient, as compared to a mean of 2.0 isolates per patient in children who had tonsillectomy for recurrent tonsillitis, idiopathic hyperplasia, or recurrent tonsillitis with hyperplasia. However, 70% of these normal control tonsils yielded at least one microorganism, with some of these including *Hemophilus influenzae, Staphylococcus aureus*, and group A β-hemolytic *Streptococcus*.

I therefore think that Brook and Foote's findings should be interpreted with caution, as there are other studies that they did not cite in their references that come out with much different information. I think the definition of what a "normal" tonsil is is of key importance in studying this problem. However, I think further study is needed. Dr Brook and his colleagues have been leaders in the field and have brought our attention to many of the new concepts regarding the microbiology of tonsillar disease that go beyond the classically accepted group A β-hemolytic *Streptococcus* as the sole cause of acute and chronic tonsillar disease.

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To the Editor:

Dr Brodsky's letter points out several important issues relating to the microbiology of "normal" tonsils. We do not believe that the data by Brodsky et al[1,2] are significantly different from ours.[3] Like Brodsky et al, we were able to recover potential pathogens such as *Streptococcus pyogenes, Staphylococcus aureus*, and *Hemophilus* species from "normal" tonsils. The tonsils we studied were not hypertrophied and did not physically obstruct the airway. Their weights were less than 3.5 g each. The only indication for their removal was speech impairment associated with velopharyngeal insufficiency. They therefore were as "normal" as was possibly feasible to obtain. It should be noted that the weight of the recurrently inflamed tonsils in Dr Brodsky's study was greater than 4 g for each tonsil. Unfortunately, we studied only four "normal" tonsils because tonsillectomy due to velopharyngeal insufficiency is rarely done, and we wanted to avoid biopsies of normal tonsils from patients who undergo surgery for other reasons.

The recovery of more aerobic organisms per tonsil in our study in both normal as well as recurrently infected tonsils may be due to methodological differences and to specimens obtained from the cores of totally removed tonsils, from which a true deep core culture was obtained.

We agree with Dr Brodsky that more work is needed to ascertain the microbiology of normal and inflamed tonsils. We also believe that for a more complete appreciation and understanding of this topic, cultures should be obtained for both aerobic and anaerobic bacteria.

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BOOK REVIEWS

Surgery of the Eyelids and Orbit — An Anatomical Approach

Bradley N. Lemke and Robert C. Della Rocca. Hard cover, illustrated, indexed, 322 pages, 1990. Appleton and Lange, Norwalk, Conn, $150.

This book is a text on ophthalmic plastic and reconstructive surgery. In reviewing it, we must ask if it offers useful information to otolaryngologists. As the authors state in the preface, the text follows an anatomic outline rather than one based on disease entities.

Section I involves the study of the bony structures of the orbit and tends to focus on orbital trauma. As in all sections, the text is concise and maintains the reader's attention. The accompanying photographs often have corresponding drawings that enhance identification of the structures being discussed. The anatomic details are good; the grasp of anatomy is obviously the authors' forte. Management of tripod fractures, discussed in a subsection, is dated by the mention but lack of demonstration of miniplate fixation. For those who frequently deal with orbital trauma, this section overall does not offer any insight not available in other recent atlases and standard texts.

Section II, covering the nose and paranasal sinuses, is likely to be of much greater use to an ophthalmologist learning about otolaryngology than in teaching an otolaryngologist. The discussion of sinonasal disease and the anatomic demonstration and brief outline of surgical techniques offer no new information.

The lacrimal system is addressed in Section III, with sections on lacrimal probing and dacryocystorhinostomy offering very detailed illustrations.

Brow lift and blepharoplasty are covered in Section IV, but not in the detail or with the clarity found in some recent facial plastic books. The section on levator surgery is well written and illustrated, however.

The anatomy of the orbital contents is discussed in Section V, which is the most detailed and informative part of this book. In the latter part of this section, various surgical approaches to the orbit are illustrated. The section on the transconjunctival approach for orbital floor exploration and then lateral orbitotomy offer information not found in other textbooks covering this subject. The final part of this section is on removal of the eye and orbital contents, and rounds out a comprehensive discourse on surgery of the orbit.

All in all, this book is well written and provides good photographs with excellent correlative drawings. Its use to otolaryngologists is limited primarily by two factors: 1) traditional otolaryngologic problems are superficially covered, and little new insight is provided, and 2) traditional ophthalmologic problems are well covered, but the information and techniques presented will be used primarily by ophthalmologists. Because of this dichotomy, our final opinion is that this book will be of little use to most otolaryngologists and would not likely be a prudent purchase for the individual practitioner. On the other hand, it would certainly be a desirable addition to a reference library.

L. CLARK SIMPSON, MD
Mobile, Alabama

Facial Flaps — Biomechanics and Regional Application

Deonard M. Dzubow. Hard cover, illustrated, indexed, 166 pages, 1990. Appleton & Lane, Norwalk, Conn, $85.

Consisting primarily of illustrations, this book is an assemblage of types of flap applications to be used in facial reconstruction. To fully appreciate the logic and application of facial flaps, one should be a specialist who deals primarily with plastic surgery of the head and neck.

DENNIS G. PAPPAS, MD
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Understanding Voice Problems: A Physiological Perspective for Diagnosis and Treatment

Raymond H. Colton and Janina K. Casper, with contributions from Minoru Hirano. Hard cover, illustrated, indexed, 355 pages, 1990. Williams & Wilkins, Baltimore, Md, $34.95.

The authors of this text approach the topic of voice disorders from the perspective that one must first acquire knowledge of a patient's vocal pathophysiology and combine this with acoustic and perceptual information and an understanding of psychodynamics. The subject matter covers only those disorders that involve laryngeal structure and function, thereby excluding problems encountered by hearing-impaired or alaryngeal speakers, or individuals with resonance disorders.

Chapters dealing with anatomy and physiology are placed at the end of the volume, as is a brief chapter containing a compilation of normative data. The authors placed these topics so as to facilitate referential use. The first six chapters cover the process of differential diagnosis, laryngeal histopathology, problems associated with voice misuse and abuse, nervous system involvement, organic disease, and trauma.

Chapter 7 presents protocols for taking an in-depth voice history and for conducting an examination. The authors effectively discuss the importance of a skillful interviewing technique that allows critical information to be obtained while establishing a working relationship with the patient. Laryngeal examination procedures described include both direct and indirect methods such as laryngoscopy, use of fiberoptics, stroboscopy, and ultrasound. Also included are descriptions of acoustic measures of fundamental frequency, electroglottography, electromyography, and measurements of respiratory function. Last addressed are the familiar, noninstrumental evaluation methods involving critical listening, observation, and description.

Chapters 8 and 9 cover surgical and medical management of voice disorders, as well as vocal rehabilitation procedures commonly used by speech/language pathologists. A variety of color and black-and-white photographs, illustrations, tables, and graphs supplement the volume. Also helpful are an appendix and a well-organized and comprehensive index.

With its overall thoroughness and clarity of presentation, this text certainly accomplishes its authors' intended purpose of providing students and practitioners with a valuable source of information on the current methods of diagnosis and management of vocal disorders.

FERNE MCCLINTOCK, MS
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Pathology of the Nose and Paranasal Sinuses

H. B. Hellquist. Hard cover, illustrated, indexed, 164 pages, 1990. Butterworth & Co, London, England, $95.

This is a concise review of different pathologic disorders of the nose and sinuses. The book is divided into 12 chapters, including non-neoplastic and neoplastic disorders, anatomy, allergy and allergylike conditions, salivary gland tumors, malignant melanoma, and miscellaneous tumors and tumorlike lesions. Each chapter is subdivided into various topics accompanied by a brief text and illustrative clinical and histologic photographs. This book is well organized, well referenced, and a good albeit brief review of the selected topics. It would be of special interest to otolaryngologists and pathologists for a quick review of a broad range of nose and sinus disorders.

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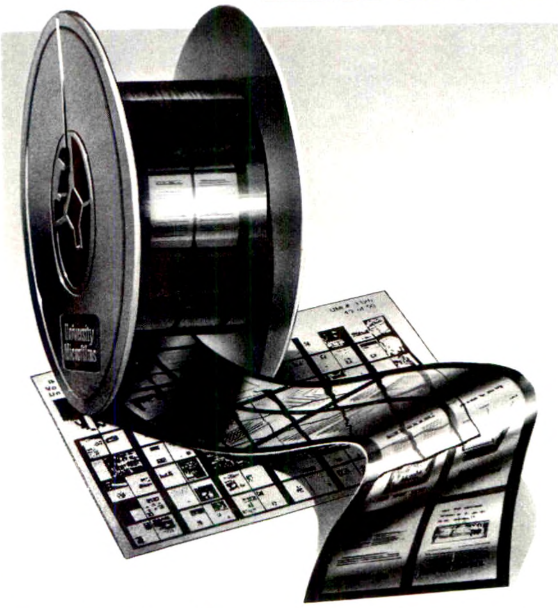

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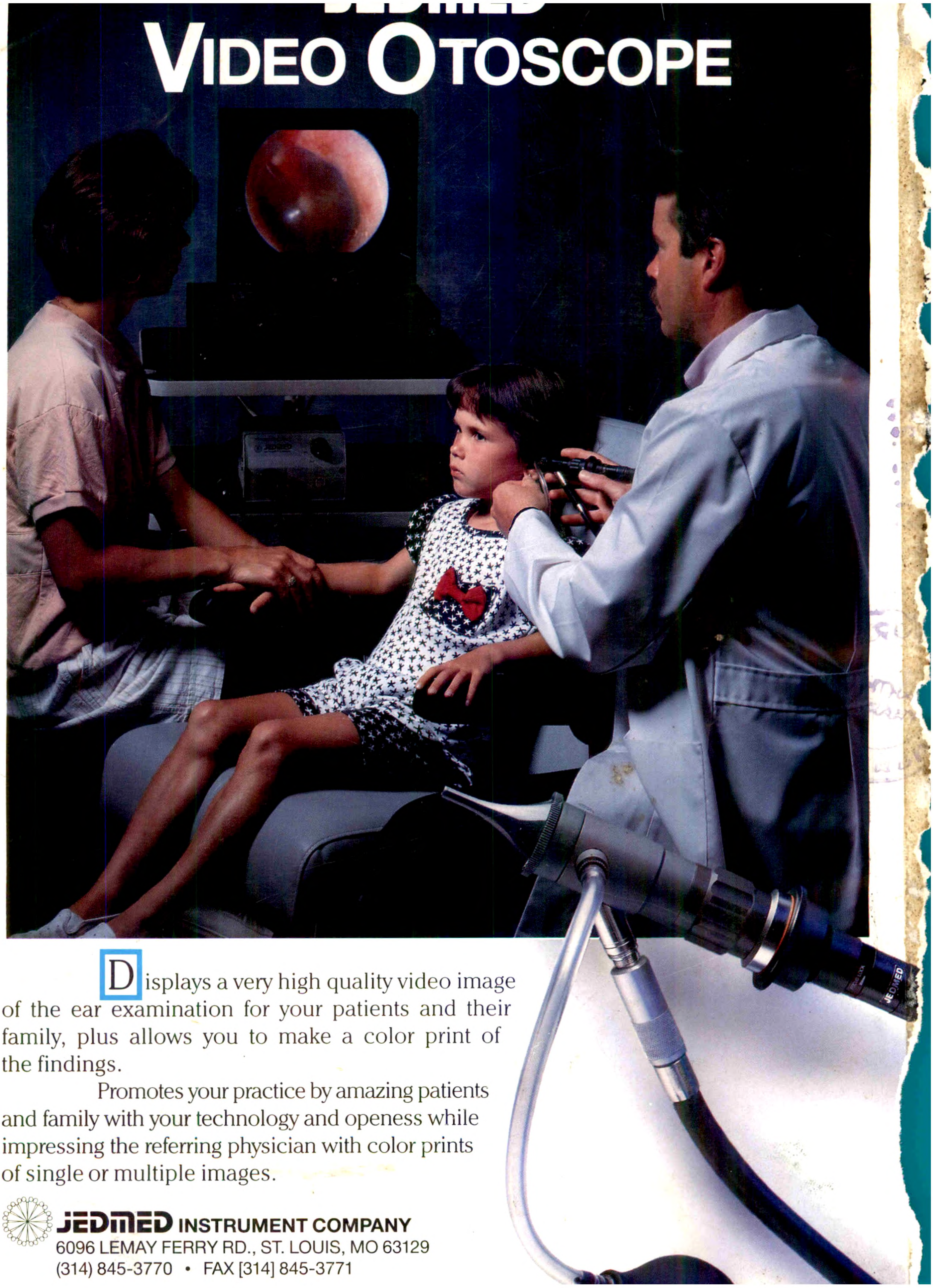

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